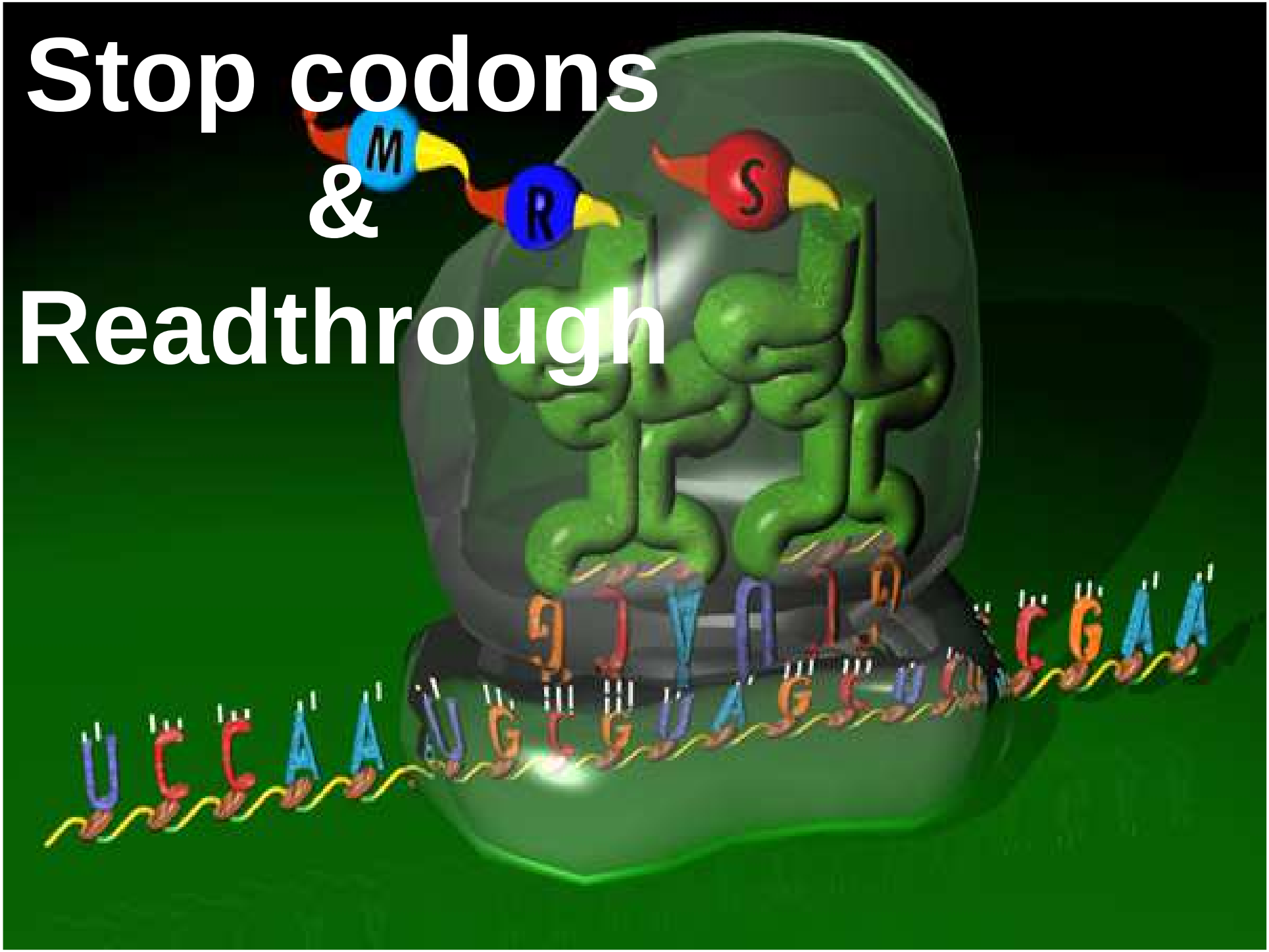
Stop codons & Readthrough



Fidelity in protein synthesis

DNA replication and transcription are based on complementarity and correctly matched base-pairing.

During translation, each tRNA is covalently bound to an amino acid in order to be accomodated in the ribosomal A-site due to correspondence of codon (on mRNA), anticodon (on tRNA), and amino acid.

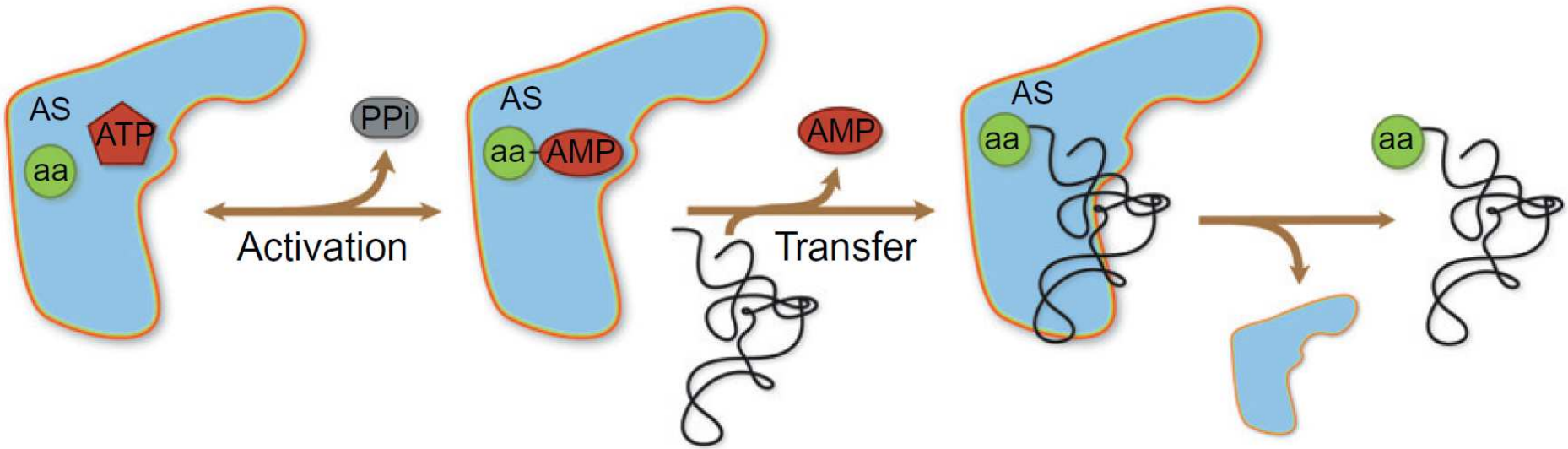
Three strategies ensure a balance between velocity (3-5 aa/sec) and accuracy (**error rate ~10^{-4}**):

1. Editing (tRNA/amino acid) ---------- Aa-tRNA synthetase
2. Kinetic proofreading (codon/anticodon) ----- Ribosome
3. Induced fit (codon/anticodon) ----------

1. Editing

Aminoacyl-tRNA synthetase

- ATP-dependent enzymes that covalently link amino acids to tRNAs
- Specific for each amino acid and for the corresponding tRNA(s)



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"Double-sieve model" (Alan Fersht, 1977):
According to this hypothesis, the synthetic active site acts as a first coarse sieve, which can bind and activate the cognate substrate as well as isosteres and smaller amino acids while rejecting larger amino acids. The editing site serves as a second fine sieve to selectively hydrolyze the isosteric amino acid but not the cognate amino acid based on size and chemical discrimination

1. Editing

Aminoacyl-tRNA synthetase

- ATP-dependent enzymes that covalently link amino acids to tRNAs
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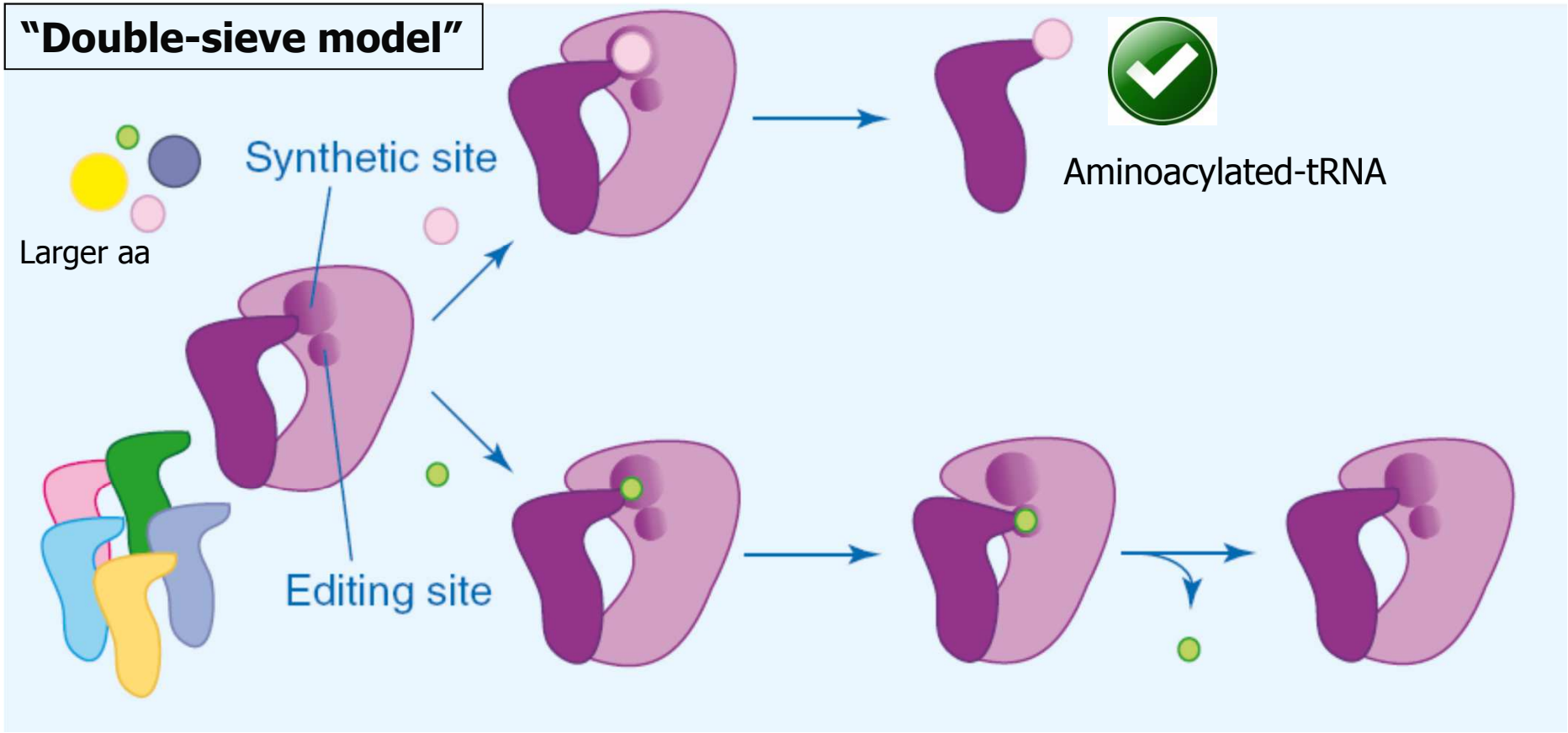
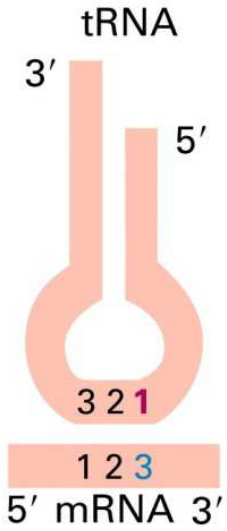


Non-standard codon/anticodon base pairing at the wobble position



If these bases are in first, or wobble, position of anticodon

C	A	G	U	I	then the tRNA may recognize codons in mRNA having these bases in third position
G	U	C U	A G	C A U	

n° aa = 20

n° tRNA/cell = <61

n° combinations = 64

(61 coding and
3 nonsense codons)

Non-standard ("wobble") base-pairing between the first anticodon the the third codon bases

1 tRNA □ more than 1 codon

1 aa □ more than 1 tRNA

Es. mRNA: UU**U**, UU**C** (Phe)
tRNA: AA**G** AA**G**

Non-standard codon/anticodon base pairing at the wobble position

If these bases are in third, or wobble, position of codon of an mRNA				
C	A	G	U	
G I	U I	C U	A G I	then the codon may be recognized by a tRNA having these bases in first position of anticodon

n° aa = 20

n° tRNA/cell = <61

n° combinations = 64

(61 coding and
3 nonsense codons)

Non-standard ("wobble") base-pairing between the first anticodon the the third codon bases

1 tRNA □ more than 1 codon

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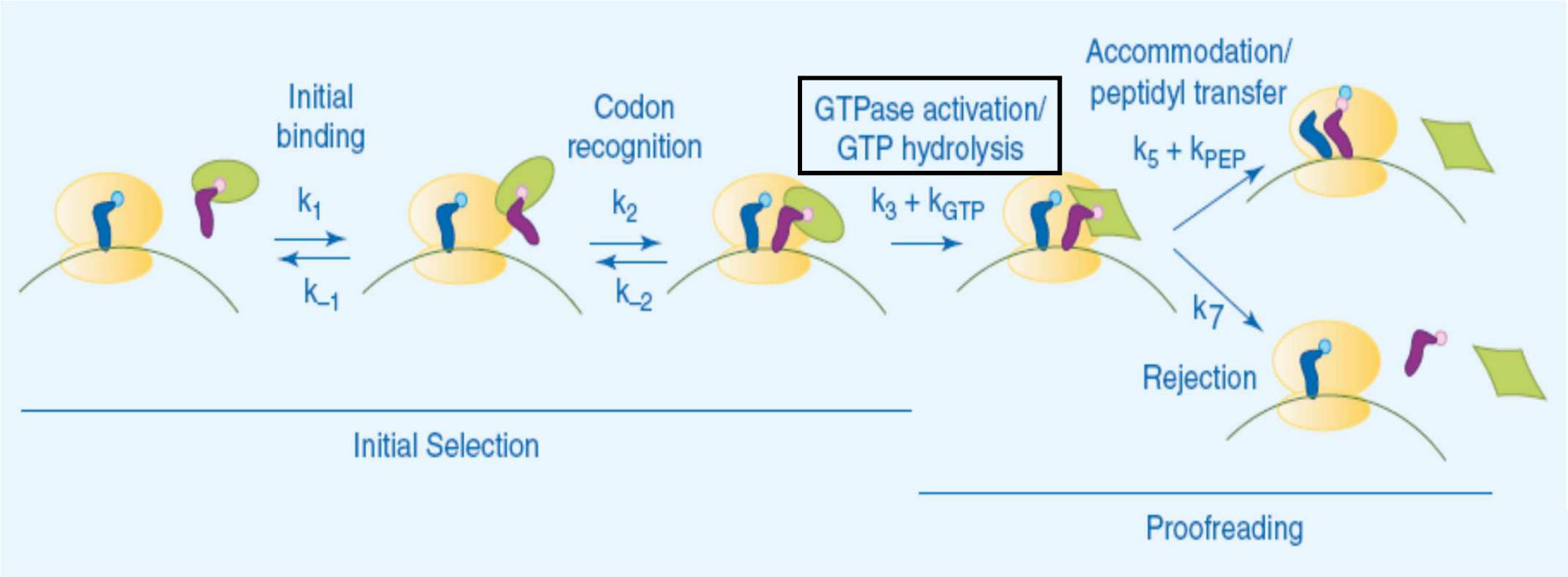
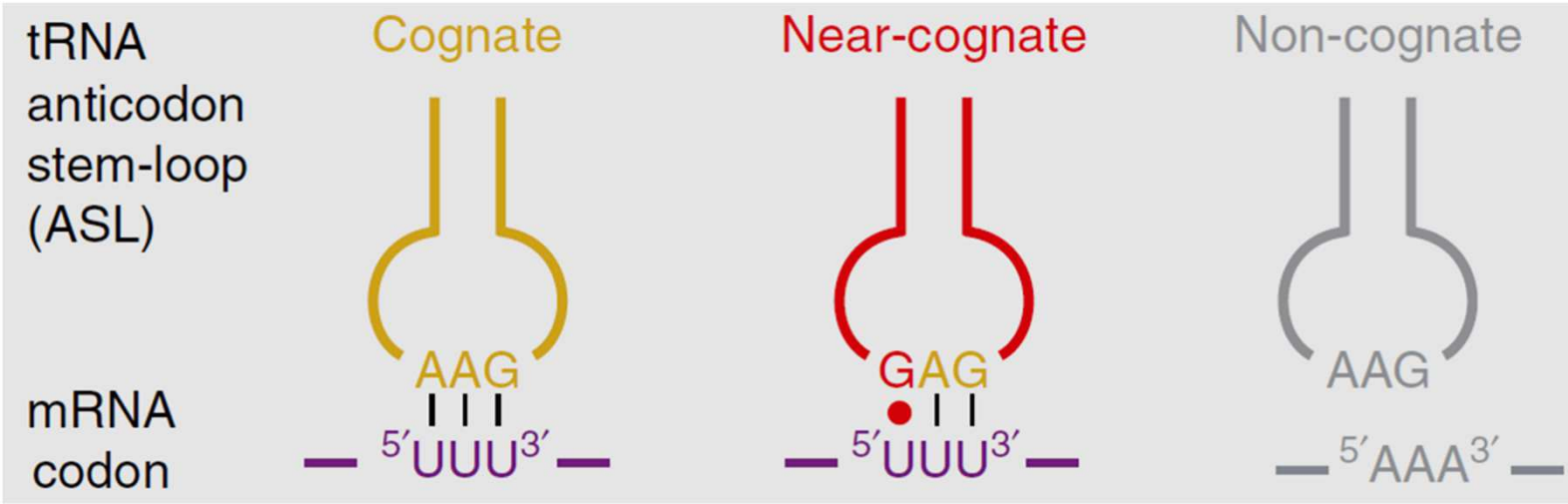
2. Kinetic proofreading





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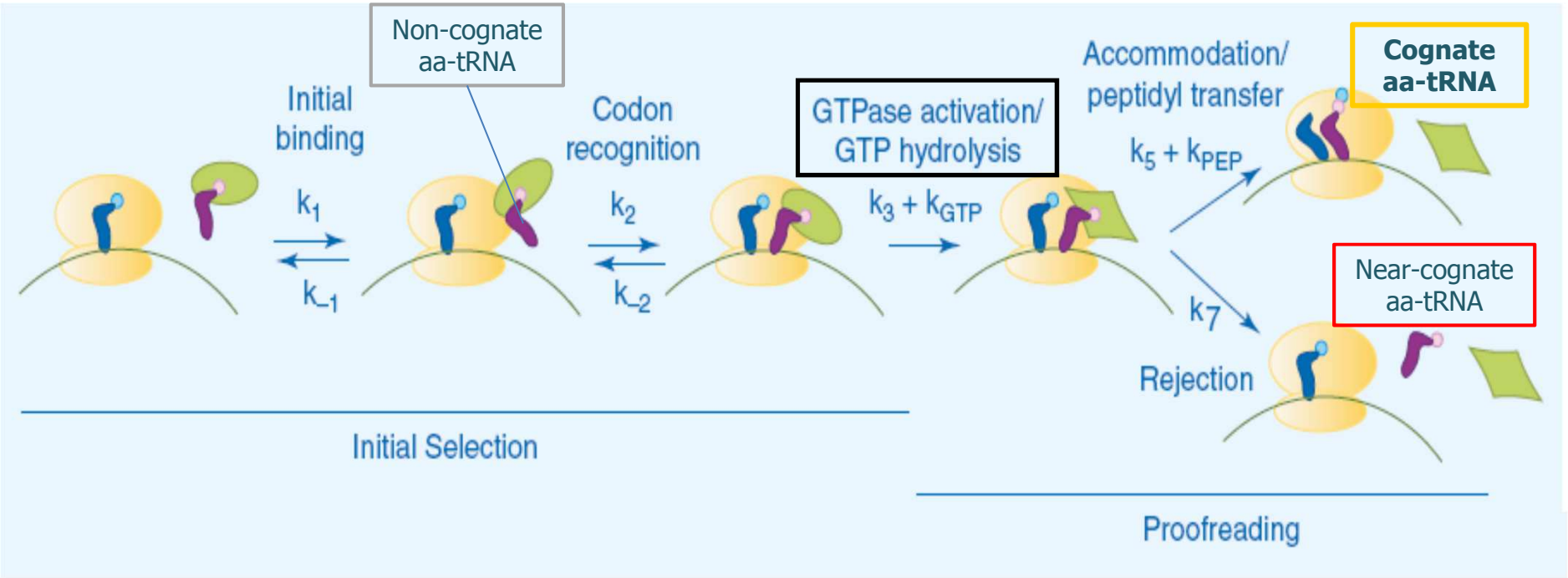

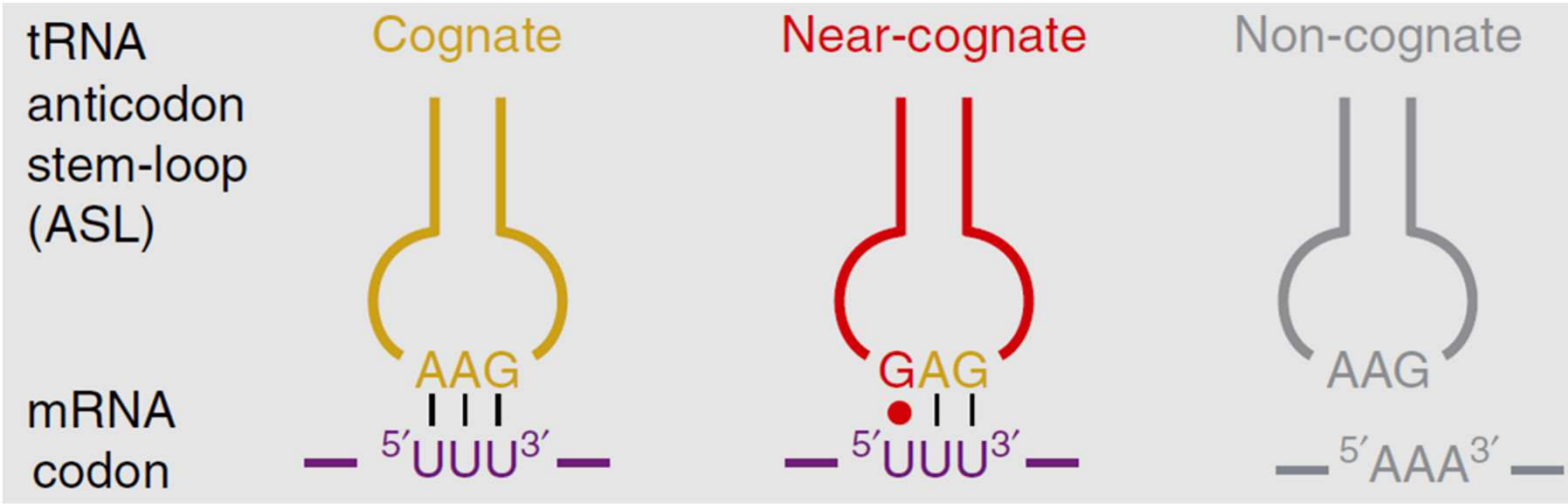

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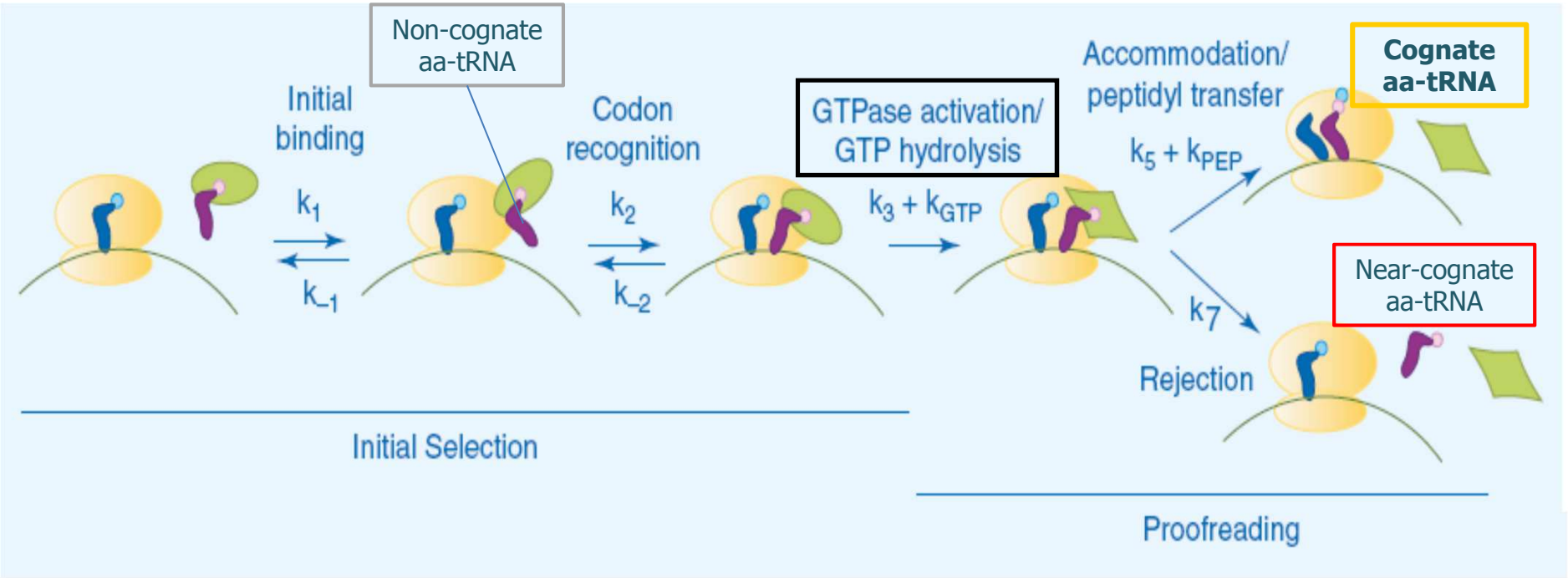

3. Induced fit

The correct aa-tRNA (named **cognate aa-tRNA**) induces a conformational change both in ribosome and tRNA (high rate of GTP hydrolysis)

The energetic cost of accomodation-induced conformational changes of ribosome and tRNA are lower for cognate aa-tRNAs.

Translational reprogrammed genetic decoding (RECODING) during protein synthesis

Recoding: regulatory mechanisms of protein expression that include several non-canonical events, opposite to the DNA → RNA → Protein central dogma of biology

Recoding was found to be associated to elongation and termination phases:

Elongation phase

- +1 Frameshifting
- -1 Frameshifting
- Ribosome hopping

Termination phase

- Stop codon **Readthrough** (frequency: $\mathbf{10^{-4}}$)

Cobucci-Ponzano *et al.*, Molecular Microbiology, 2005

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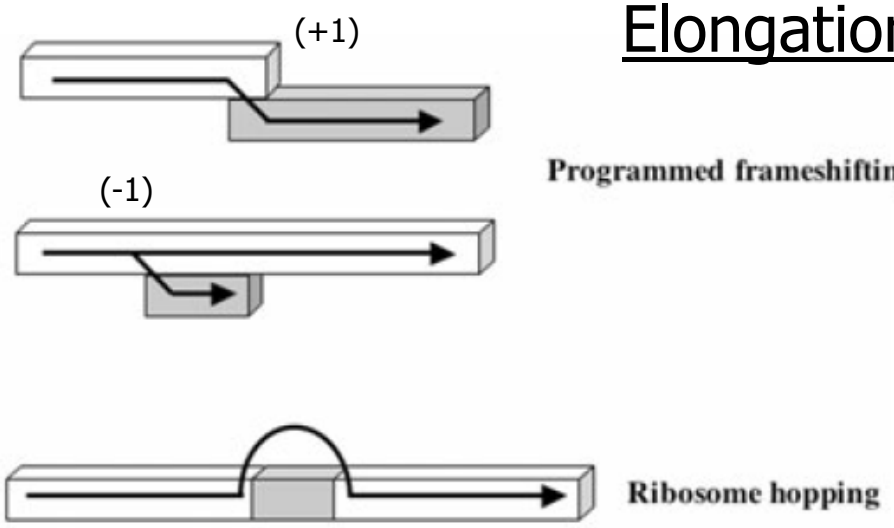


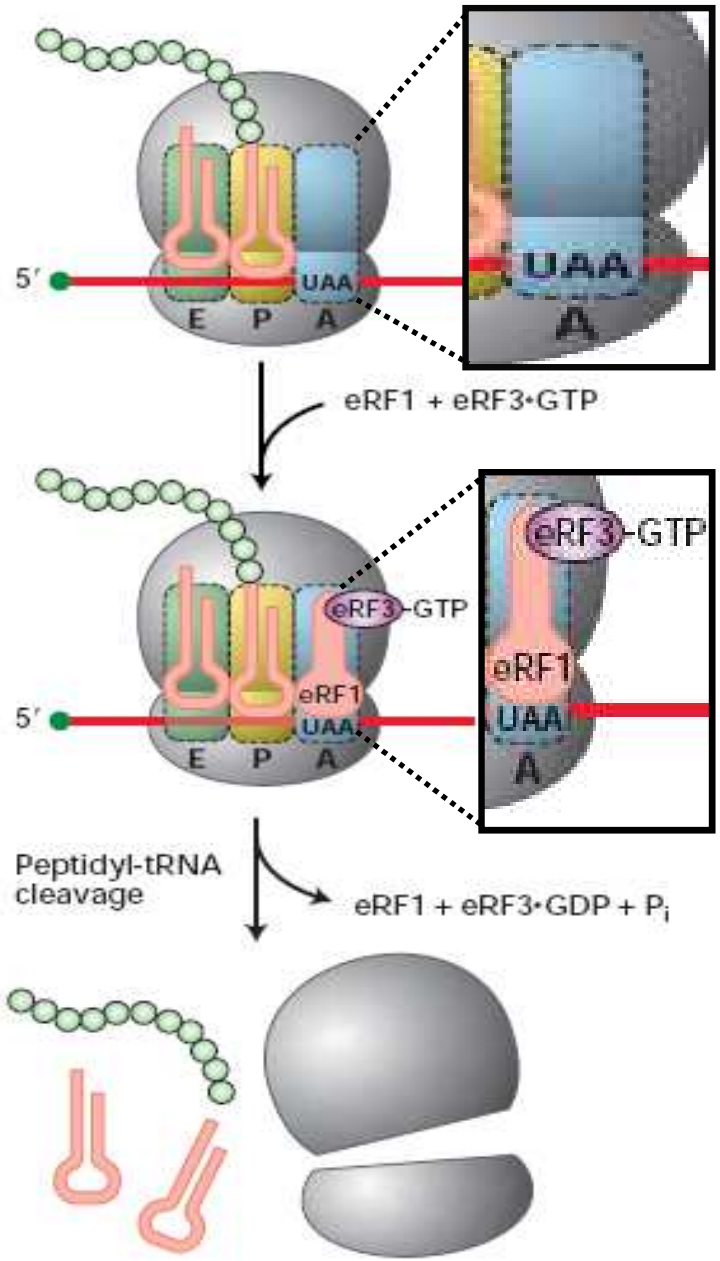
Elongation phase

Ribosomes switch to an alternative frame (±1) at a specific shift site

Ribosomes suspend translation at a certain site and then resume translation downstream

Cobucci-Ponzano *et al.*, Molecular Microbiology, 2005

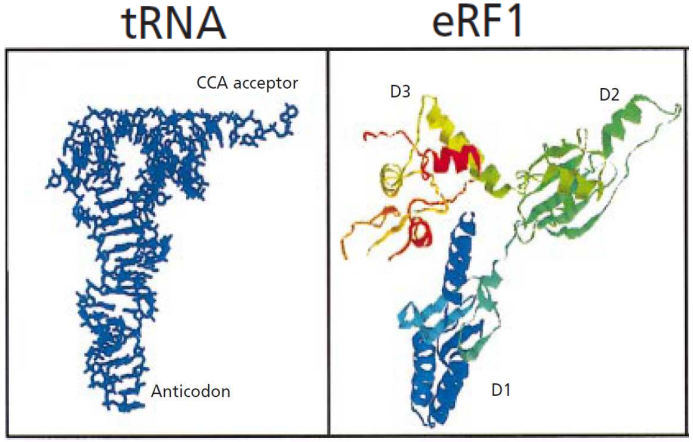
Translation termination



In-frame stop codon (UAG, UGA, UAA) enters the ribosome A-site

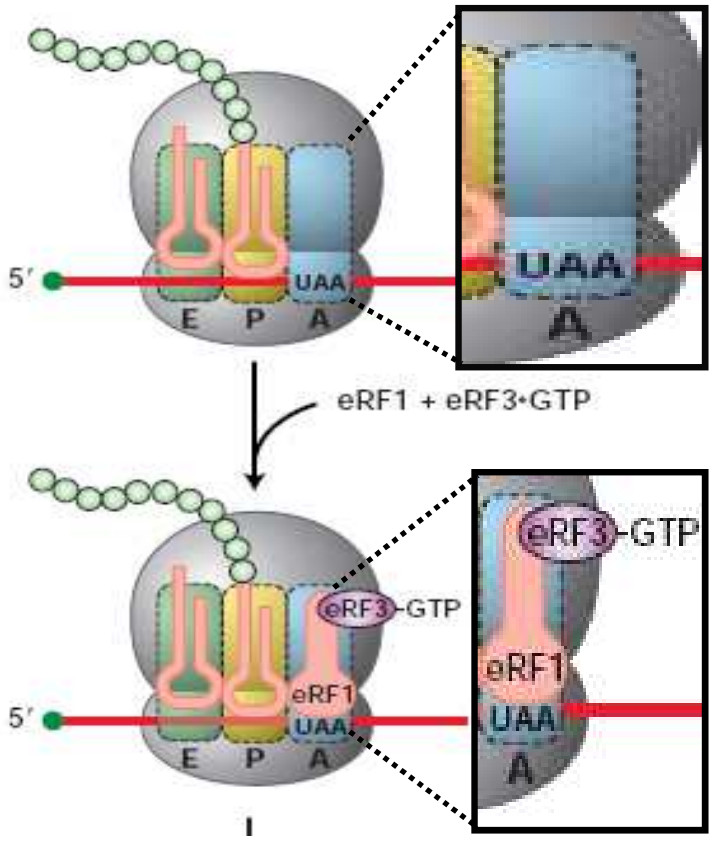
Recongition of the stop codon by release factor eRF1

tRNA mimicry and competition



Hydrolysis of the P-site-bound peptidyl-tRNA and release of the nascent polypeptide

Translation termination



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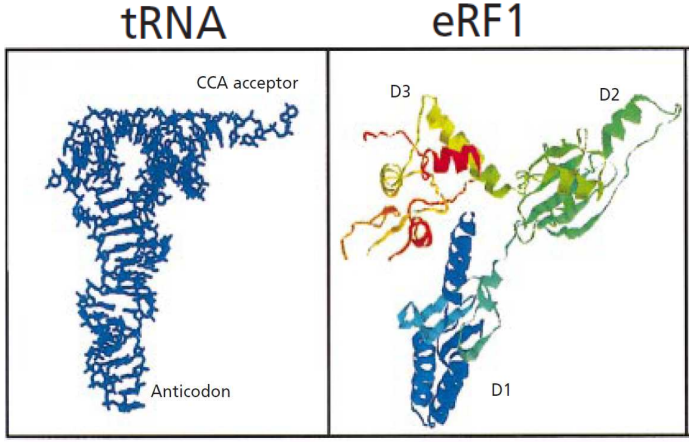


Termination phase

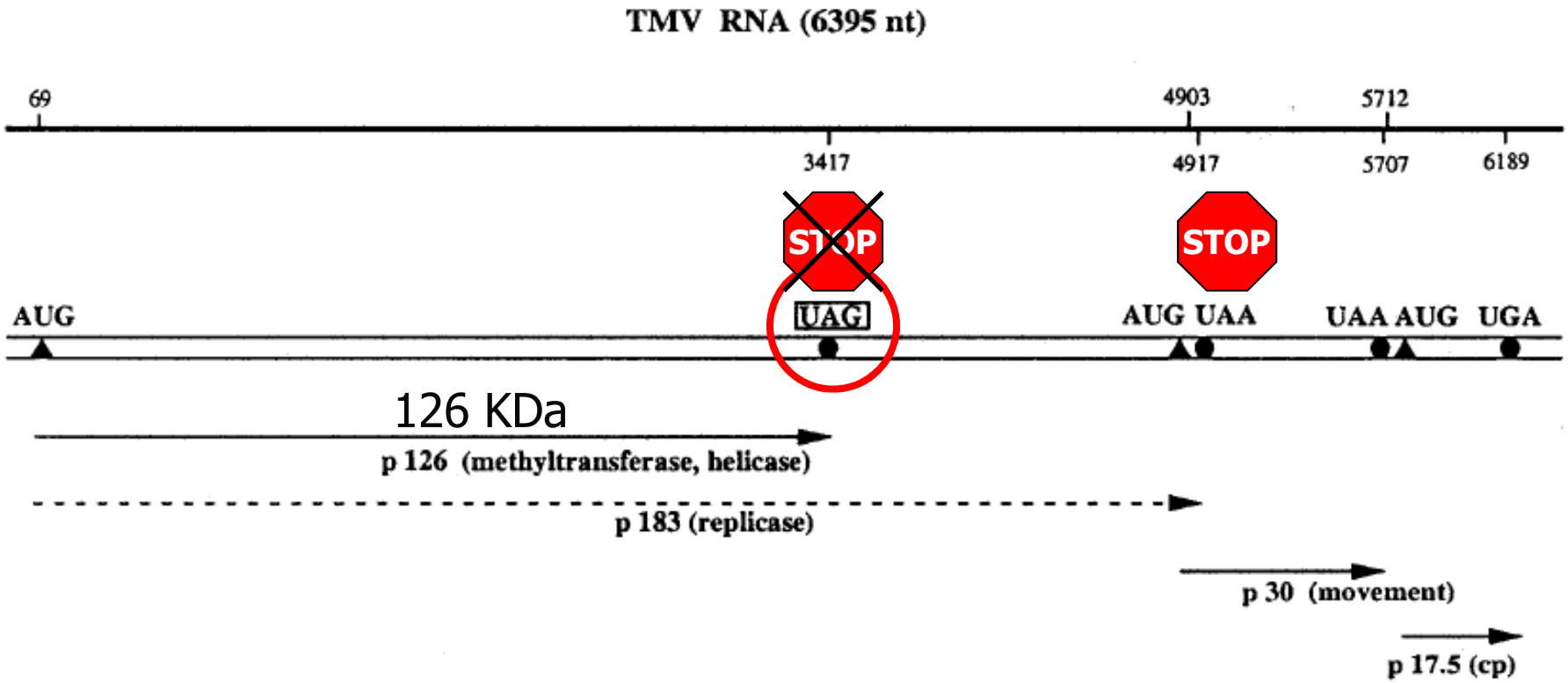
Stop codon readthrough

Translation is continued *beyond* the stop codon

Translational READTHROUGH

A regulatory mechanism of gene expression, extensively used by ssRNA viruses, which provides the differential production of more than one polypeptide from a single mRNA

Virus (small genome) → **Readthrough** → Expansion of genetic information



Beier and Grimm, Nucleic Acids Res (2001)

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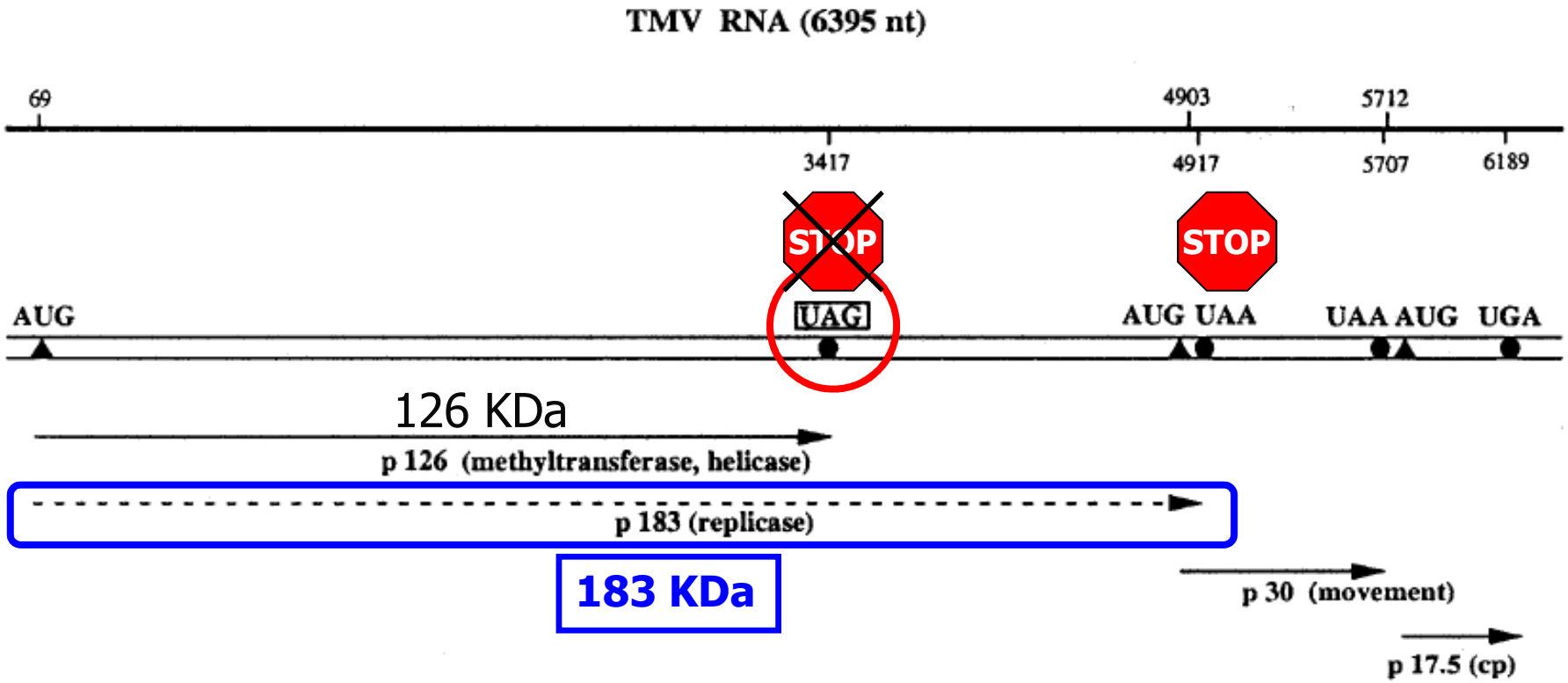


Beier and Grimm, Nucleic Acids Res (2001)

Termination codons can be "Leaky" stop signals

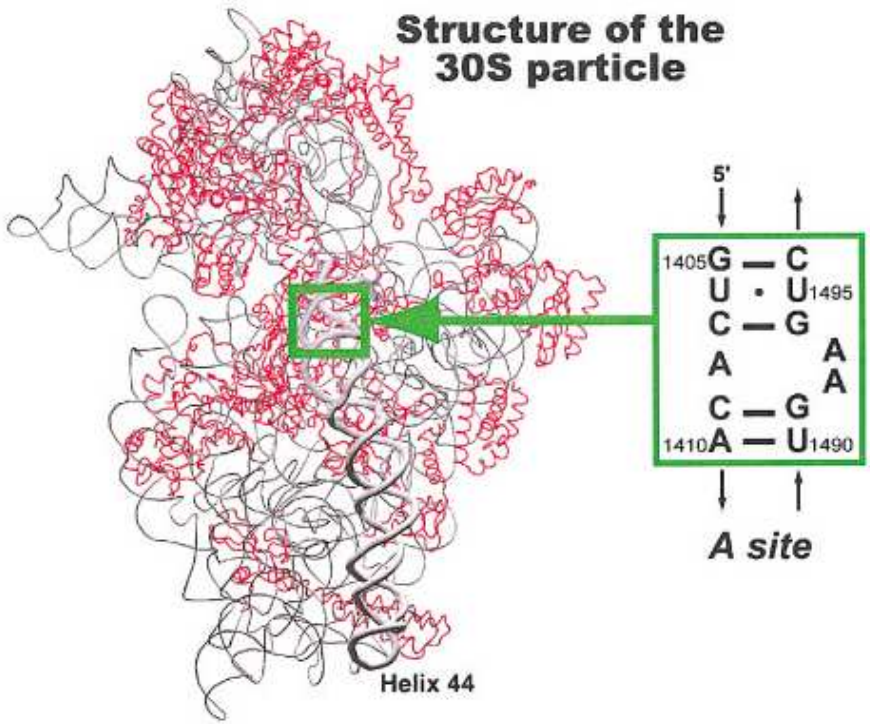
Virus	Genus	"Leaky" termination codon	Readthrough product/ function
Enterobacteria phage Qβ	Allolevivirus	UGA	Coat protein extension; assembly
Murine leukemia virus (MuLV)	Gammaretrovirus	UAG	Reverse Transcriptase
Sindbis virus (SIN)	Alphavirus	UGA	Replicase
Tomato bushy stunt virus (TBSV)	Tombusvirus	UAG	Replicase
Carnation mottle virus (CarMV)	Carmovirus	UAG	Replicase
Tobacco necrosis virus (TNV)	Necrovirus	UAG	Replicase
Maize chlorotic mottle virus (MCMV)	Machlomovirus	UAG	Replicase
Barley yellow dwarf virus (BYDV)	Luteovirus	UAG	Coat protein extension: aphid transmission
Potato leafroll virus (PLRV)	Polerovirus	UAG	Coat protein extension; aphid transmission
Pea enation mosaic virus (PEMV) RNA-1	Enamovirus	UGA	Coat protein extension; aphid transmission
Tobacco mosaic virus (TMV)	Tobamovirus	UAG	Replicase
Tobacco rattle virus (TRV) RNA-1	Tobravirus	UGA	Replicase
Peanut clump virus (PCV) RNA-1	Pecluvirus	UGA	Replicase
Soil-borne wheat mosaic virus (SBWMV) RNA-1	Furovirus	UGA	Replicase
RNA-2		UGA	Coat protein extension; fungus transmission
Potato mop-top virus (PMTV) RNA-1	Pomovirus	UGA	Replicase
RNA-3		UAG	Coat protein extension
Beet soil-borne virus (BSBV) RNA-1	Pomovirus	UAA	Replicase
RNA-2		UAG	Coat protein extension
Broad bean necrosis virus (BBNV) RNA-2	Pomovirus	UAA	Coat protein extension
Beet necrotic yellow vein virus (BNYVV) RNA-2	Benyvirus	UAG	Coat protein extension; fungus transmission
Turnip yellow mosaic virus (TYMV)	Tymovirus	UAG	Replicase extension ?

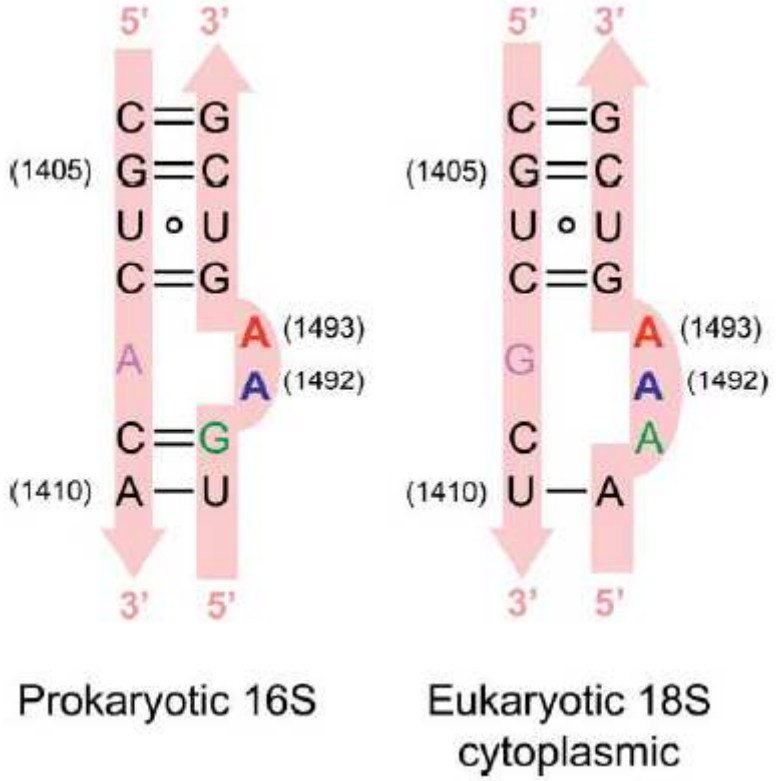
Beier and Grimm, Nucleic Acids Res (2001)

Ribosome Decoding Site

Region located within the **A-site** in the ribosomal small subunit

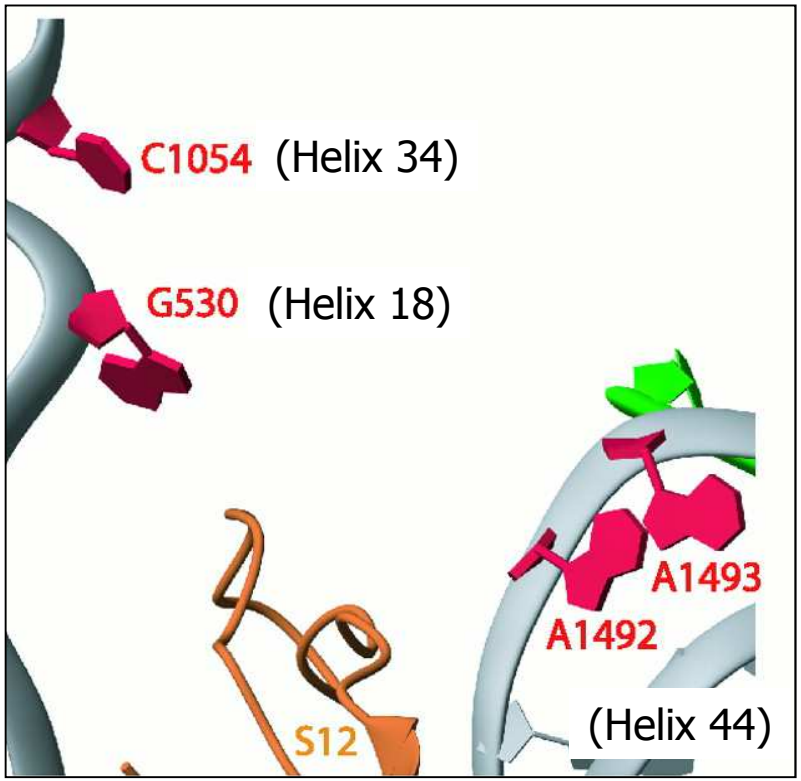
The Decoding Site contains two **adenine nucleotides (A1492 and A1493)** that **monitor codon/anticodon base pairing**



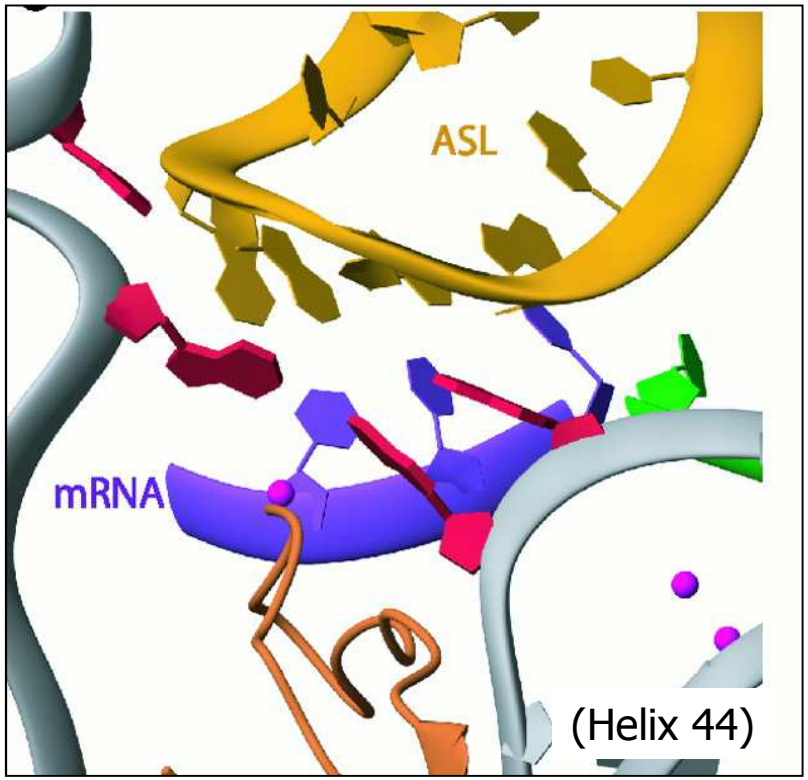


The decoding site switches to a "closed" conformation when the correct (cognate) aa-tRNA enters the A-site

"open" conformation



"closed" conformation



When the codon and a tRNA-ASL bind in the A-site, A1492 and A1493 flip out to monitor the codon-anticodon interaction

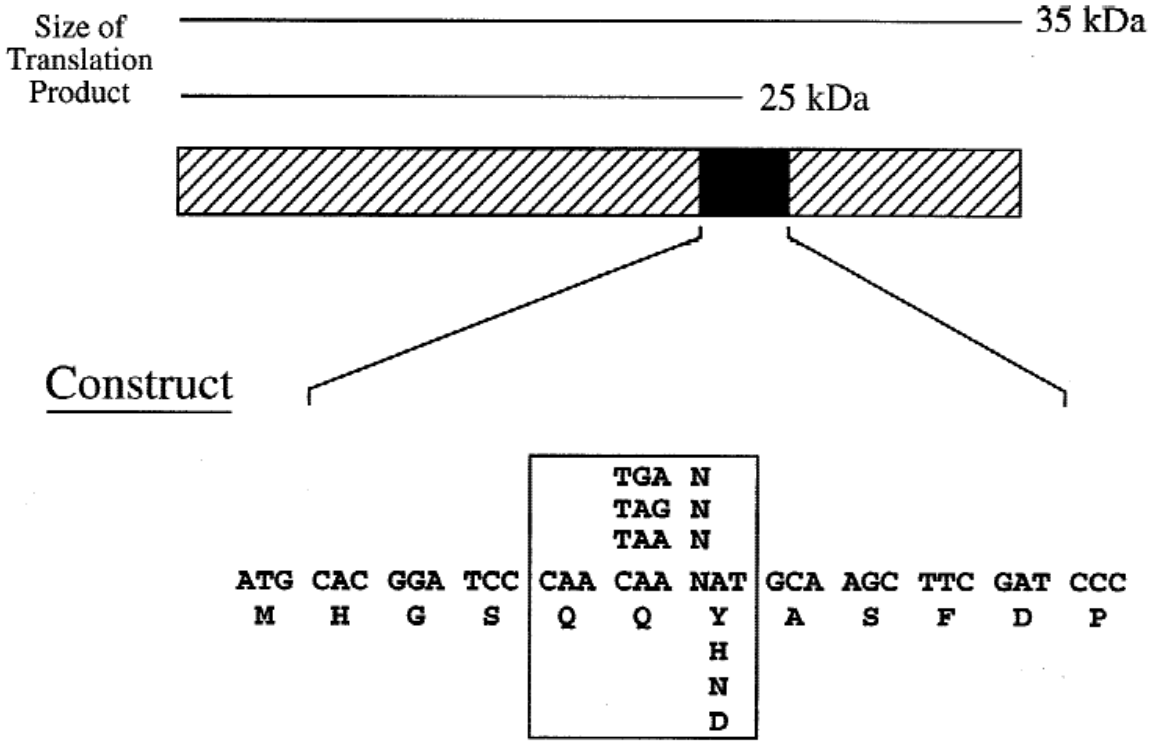
Ogle et al., Science (2001)

RNA (2000), *6*:1044–1055. Cambridge University Press. Printed in the USA.

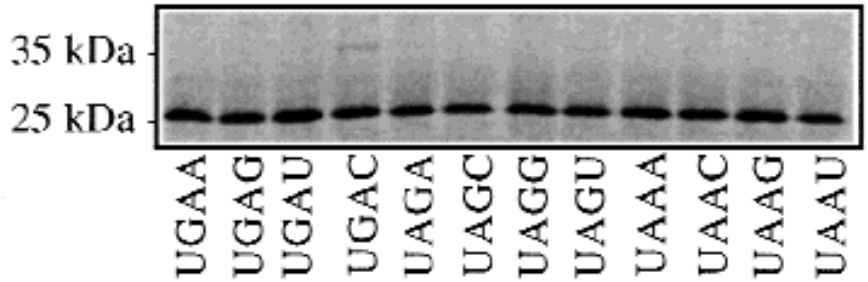
Aminoglycoside antibiotics mediate context-dependent suppression of termination codons in a mammalian translation system

MARINA MANUVAKHOVA,[1,3] KIM KEELING,[2] and DAVID M. BEDWELL[1,2]

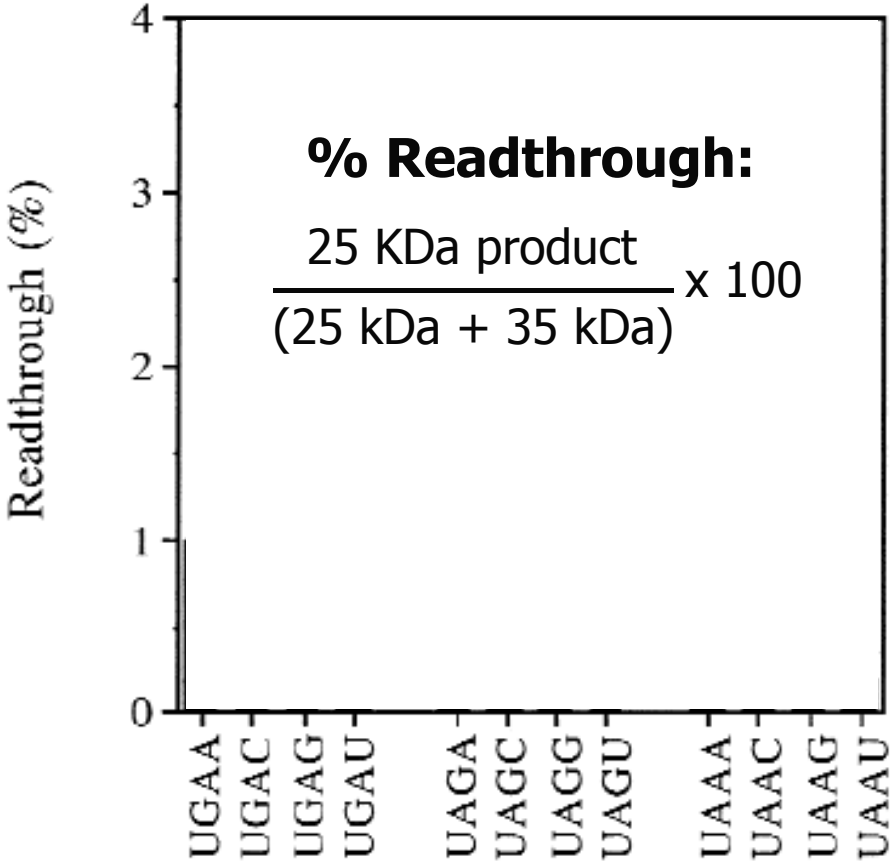
Experimental model: synthetic constructs bearing different termination signals are translated in-vitro in the presence of [^{35}S]-Met/Cys and resulting [^{35}S]-labeled polypeptides are analyzed by SDS-PAGE



Readthrough is influenced by the sequence context



The termination signal is recognized as an extended **tetranucleotide** comprised of the stop codon and the **first nucleotide** that follows



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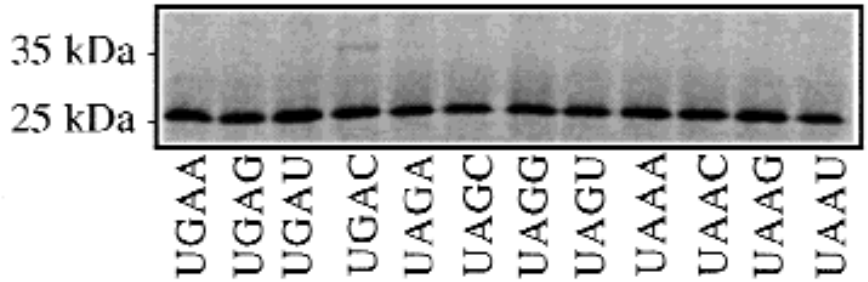

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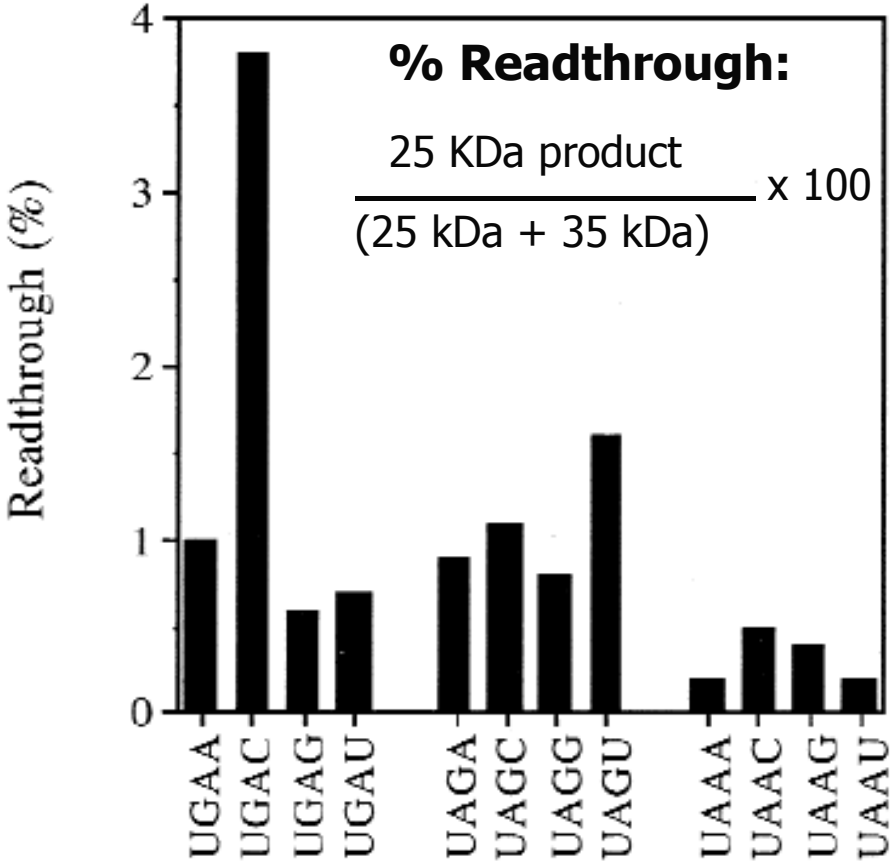

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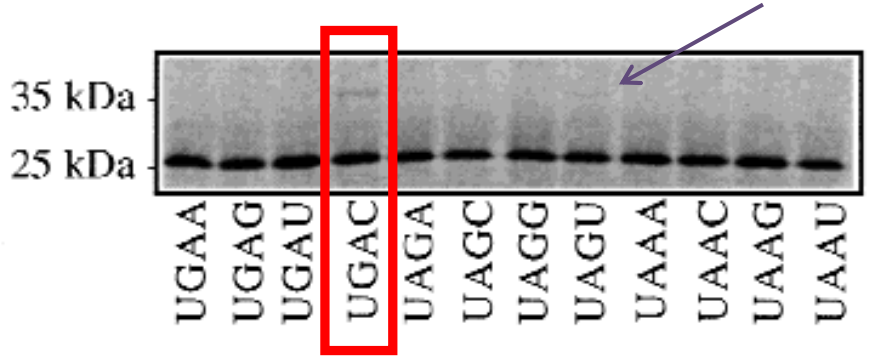
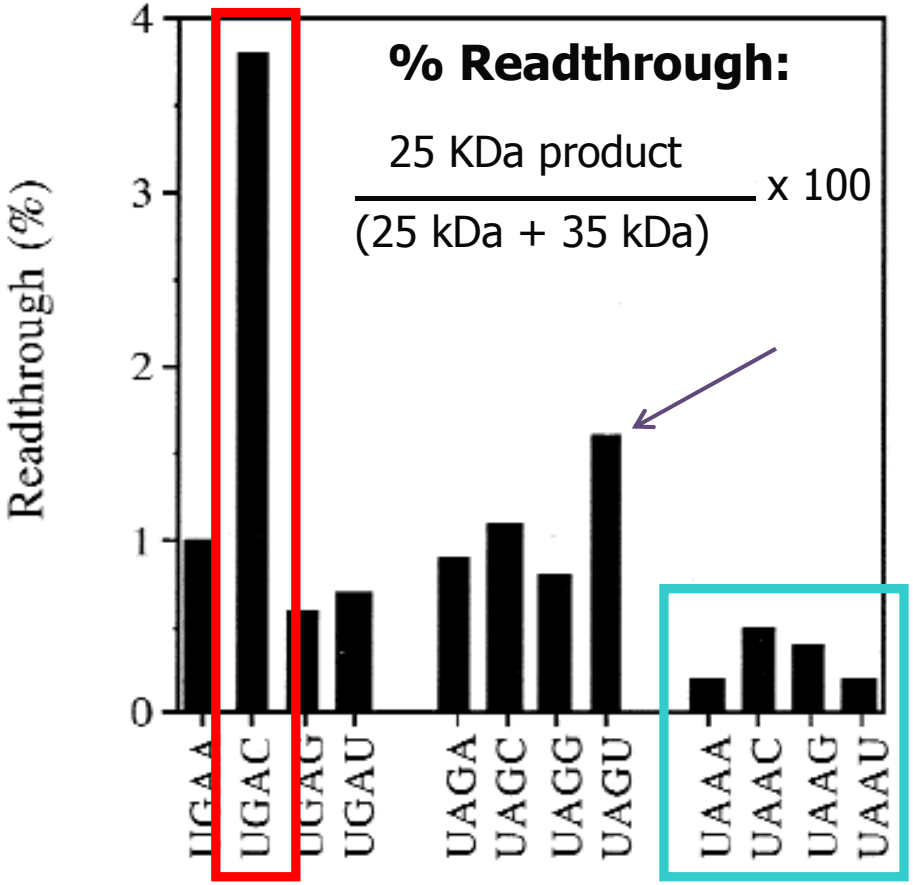


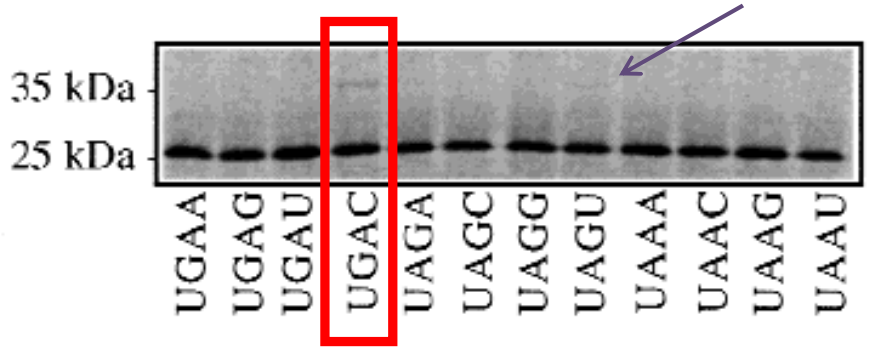


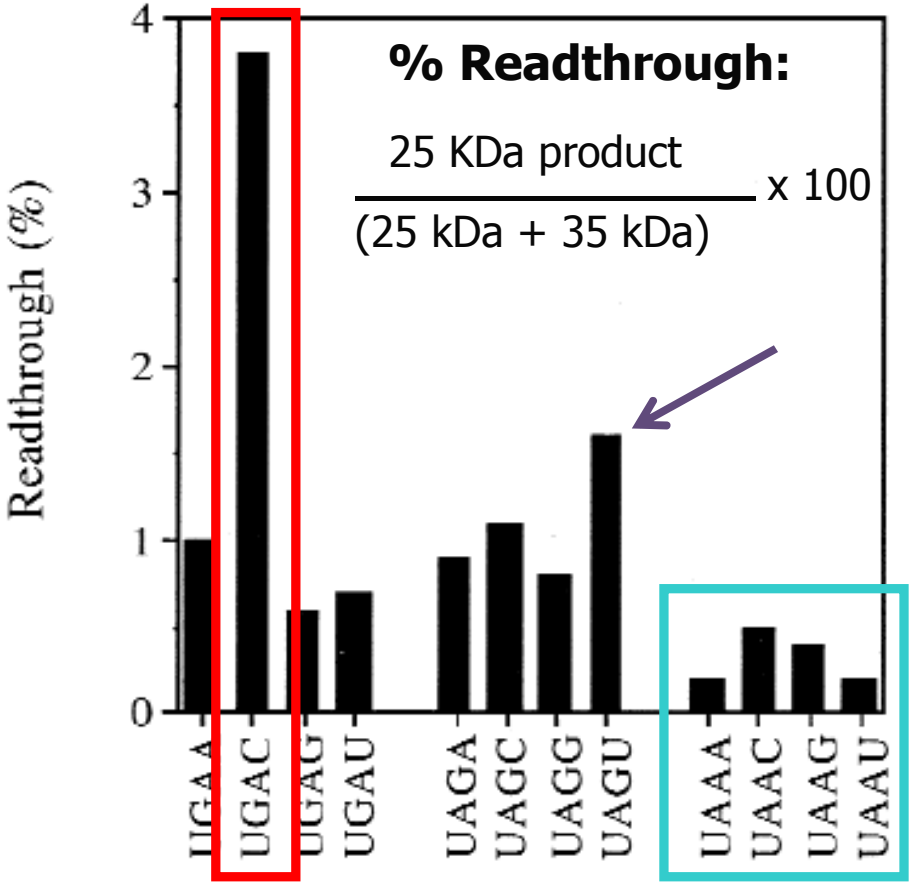
The termination signal is recognized as an extended **tetranucleotide** comprised of the stop codon and the **first nucleotide** that follows

Basal Readthrough:

UGA-C	3-4%	**Min** termination efficiency
UAG-U	1.6%	Intermediate efficiency
UAA(N)	~0.5%	**Max** termination efficiency

Readthrough is influenced by the sequence context





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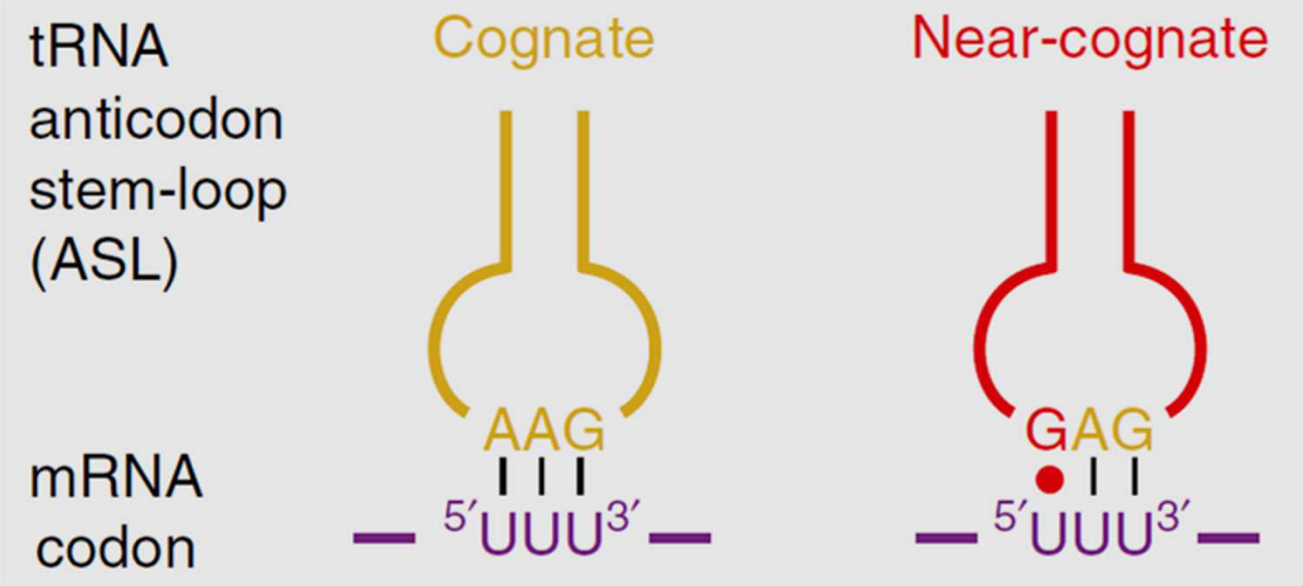
Termination efficiency

UAA > UAG ≥ UGA

READTHROUGH

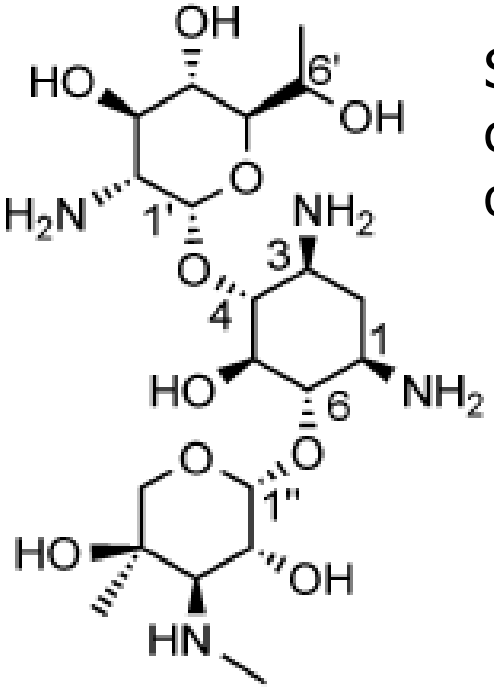
The occurrence of a basal readthrough prepares the ground for the use of molecules that are able to decrease the efficiency of translation termination, thus increasing the efficiency of readthrough itself



Aminoglycosides

A group of molecules belonging to the class of antibiotics

Aminoglycosides bind the decoding site within the A-site in the ribosomal small subunit



Geneticin (G418)

Mechanism of action

Stabilization of the decoding site (A1492 and A1493) in a conformation similar to that induced by the incorporation of a cognate tRNA (loss of proofreading capacity)

They alter the capacity of ribosome to discriminate between cognate and near-cognate aa-tRNA, thus leading to mis-incorporation of amino acids instead of binding release factors

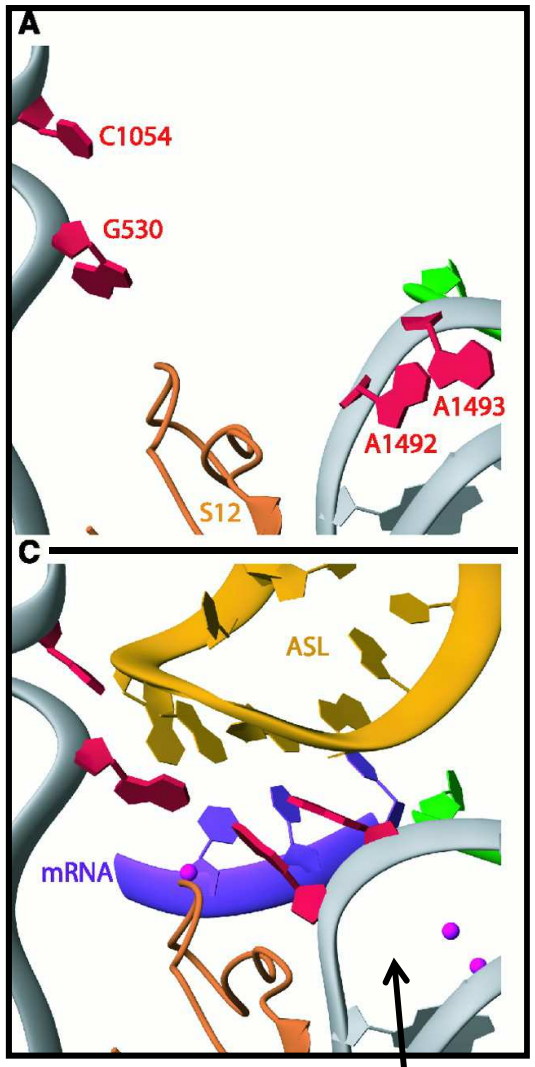
near-cognate tRNA ≈ cognate tRNA

Aminoglycosides bind the decoding site and reduce ribosome fidelity

Normal

- tRNA

- mRNA



+ tRNA

+ mRNA

Decoding site

Ogle et al., Science (2001)

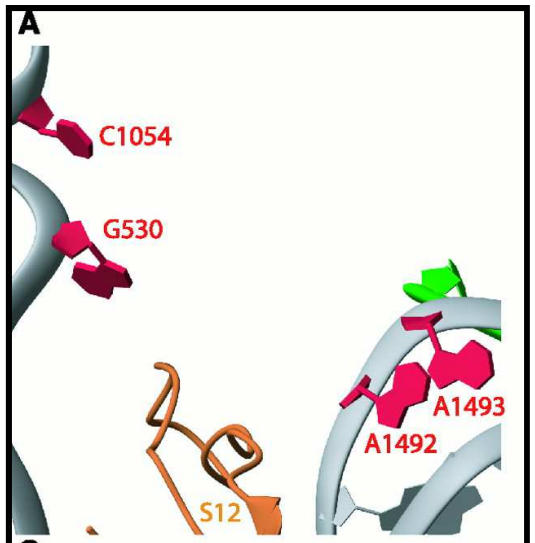
Aminoglycosides bind the decoding site and reduce ribosome fidelity

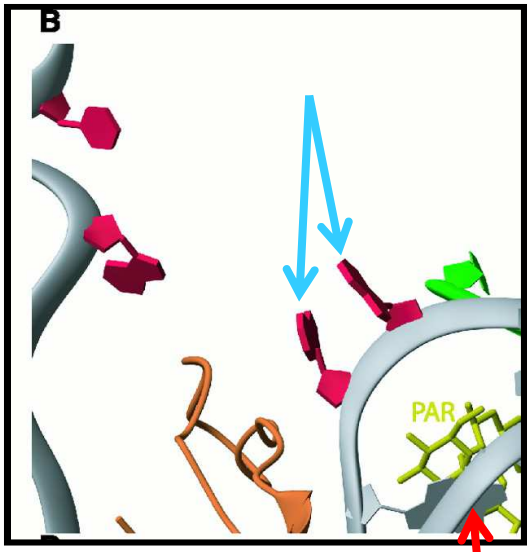
Normal

Aminoglycoside- bound

- tRNA

- mRNA

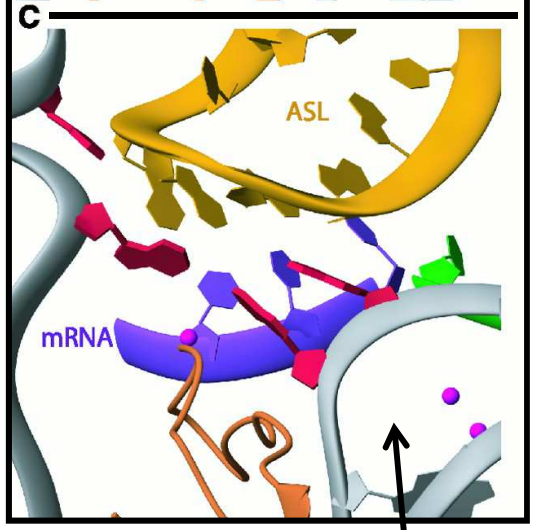




Aminoglycoside-bound
Decoding site

+ tRNA

+ mRNA



Decoding site

Ogle et al., Science (2001)

Aminoglycosides bind the decoding site and reduce ribosome fidelity

Normal

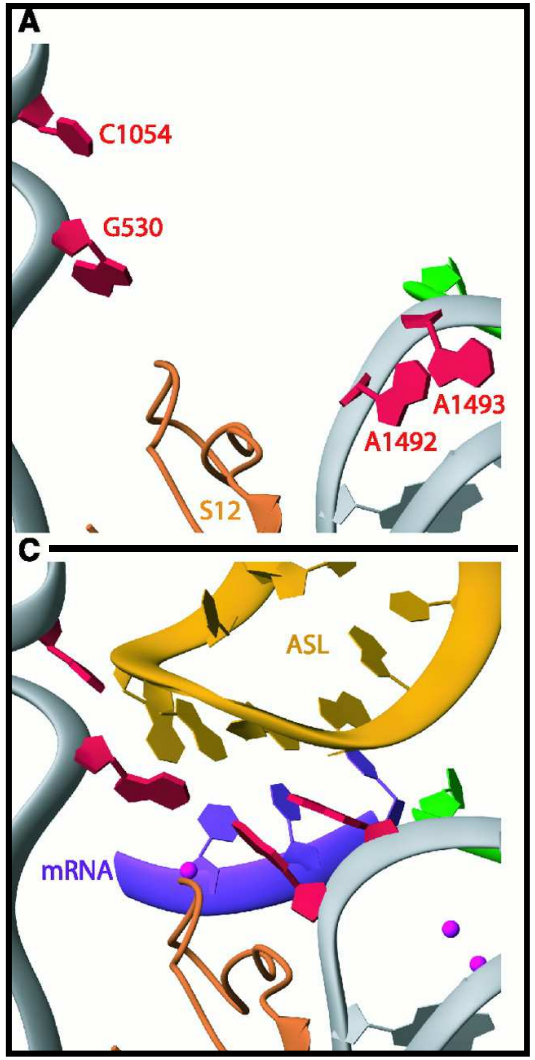
Aminoglycoside- bound

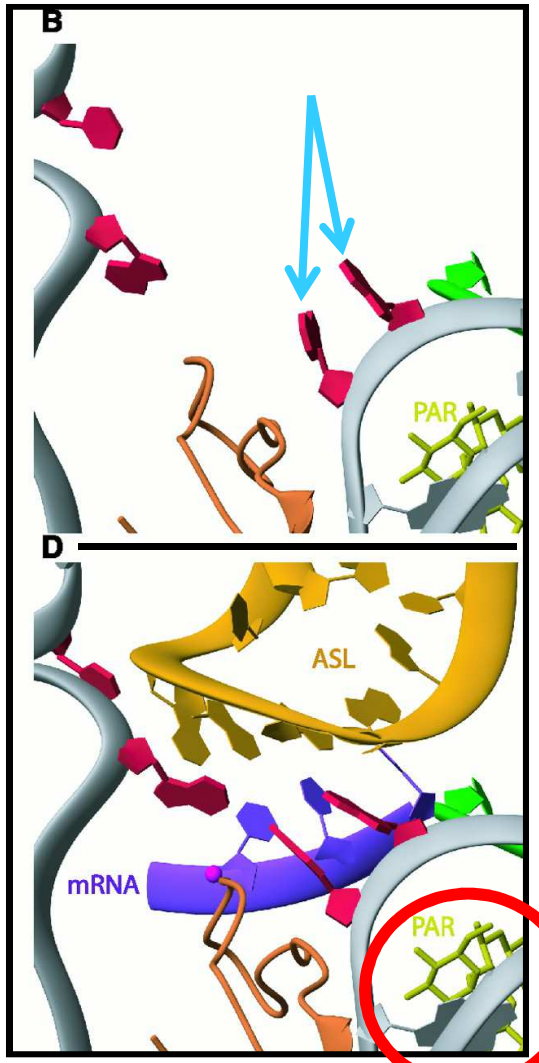
- tRNA

- mRNA

+ tRNA

+ mRNA



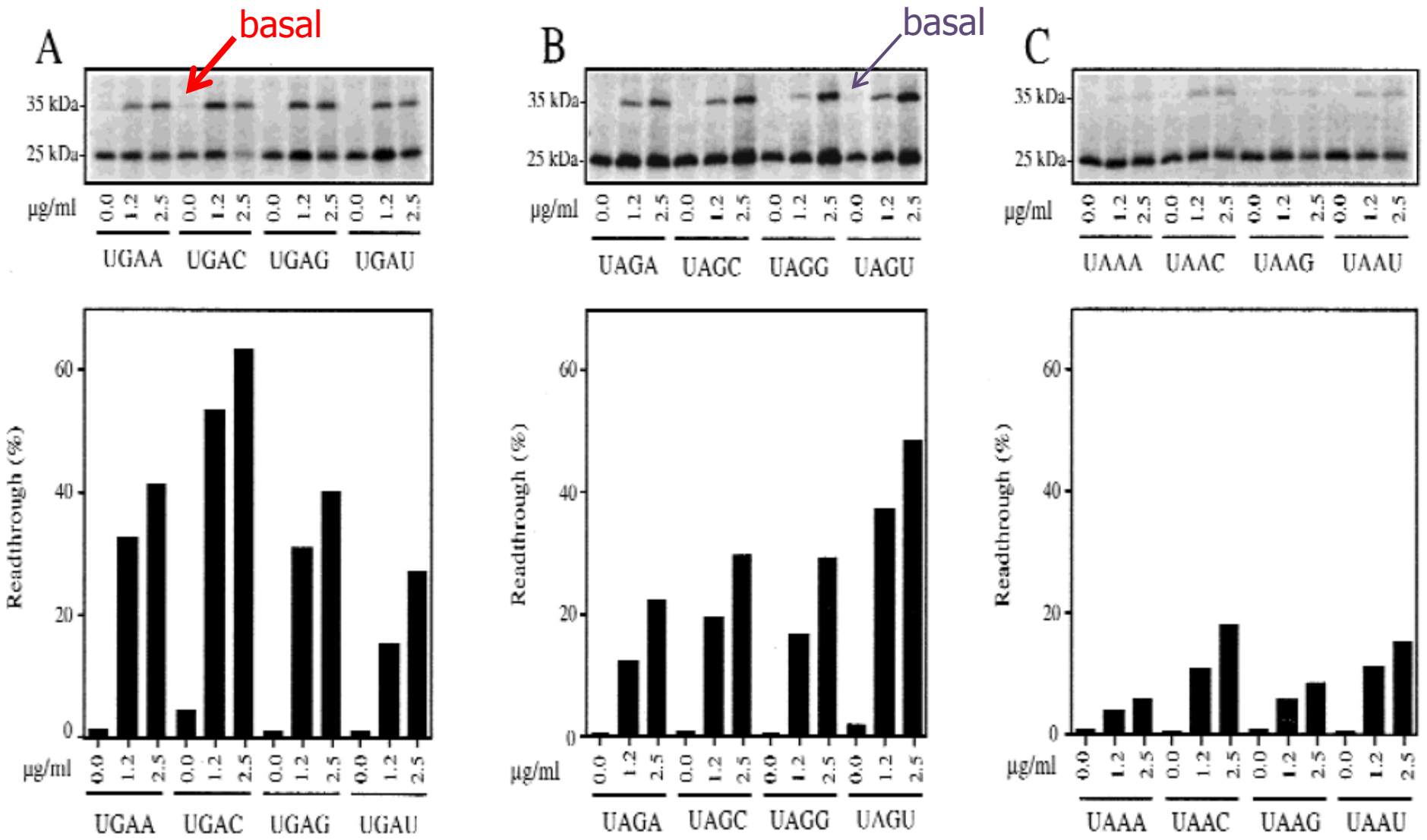


near-cognate tRNA ≈ cognate tRNA

Ogle et al., Science (2002)

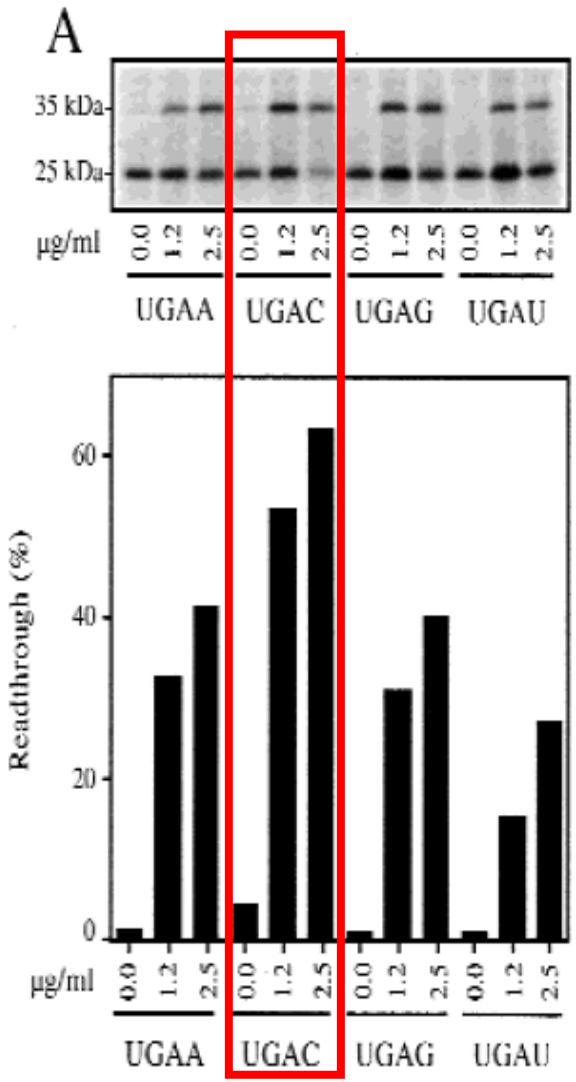
Treatment with aminoglycosides (G418) enhances readthrough

The context and/or stop codon with the highest basal readthrough (**UGAC** or UGA in general) display the most efficient G418-induced readthrough.



Treatment with aminoglycosides (G418) enhances readthrough

The context and/or stop codon with the highest basal readthrough (**UGAC** or UGA in general) are those with the most efficient G418-induced readthrough.



Basal:

UGAC 3-4%

vs

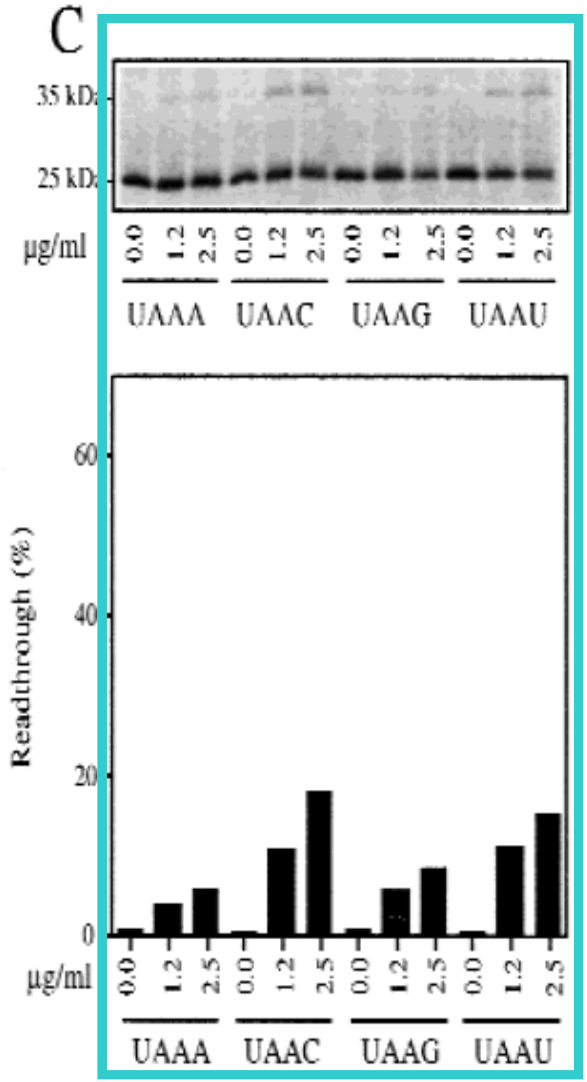
UAA(N) ~ 0,5%

G418-induced:

UGAC 63%

vs

UAA(N) 6-18%



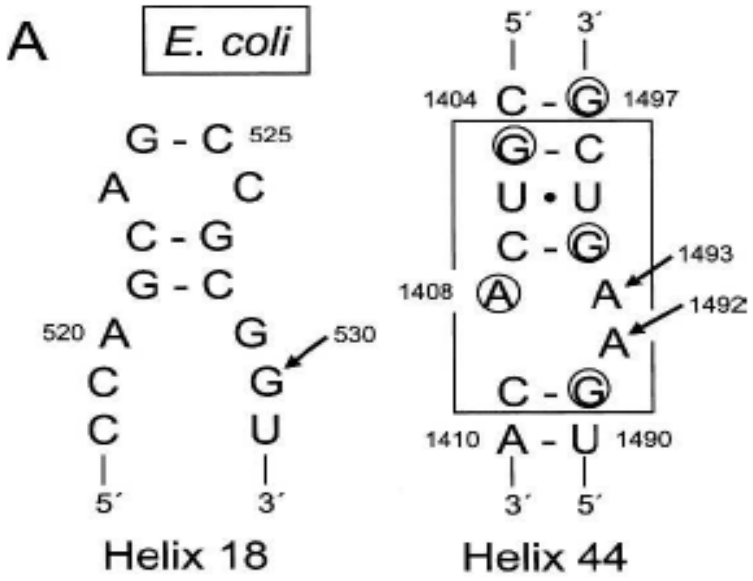
Eukaryotic ribosomal RNA determinants of aminoglycoside resistance and their role in translational fidelity

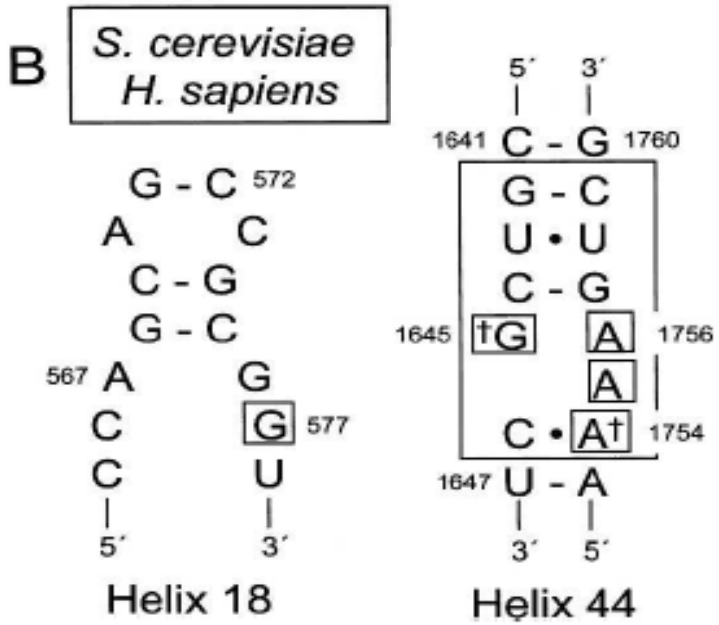
HUA FAN-MINOGUE[1,3] and DAVID M. BEDWELL[1,2]

RNA (2008), 14:148–157. Published by Cold Spring Harbor Laboratory Press.

Mutagenesis-based analysis of yeast (*S. cerevisiae*) decoding site

Mutagenesis of yeast decoding site





Mutagenesis analysis of nucleotides involved in codon/anticodon monitoring (universally **conserved**)

E. coli		*H. sapiens*/yeast
A^{1493}	--------	A^{1756}
A^{1492}	--------	A^{1755}
G^{530}	--------	G^{577}

Mutagenesis analysis of nucleotides involved in aminoglycoside binding (**non-conserved**)

E. coli		*H. sapiens*/yeast
G^{1491}	----//--	A^{1754}
A^{1408}	----//--	G^{1645}

(Fan-Minogue and Bedwell, RNA (2008)

Mutagenesis or deletion of conserved nucleotides

E. coli residue	*S. cerevisiae* mutation	Viability[a]
WT	WT	Viable (++)
G530	G577A	Lethal
	G577C	Lethal
	G577U	Lethal
	G577Δ	Lethal
A1492	A1755G	Lethal
	A1755C	Lethal
	A1755U	Lethal
	A1755Δ	Lethal
A1493	A1756G	Lethal
	A1756C	Lethal
	A1756U	Lethal
	A1756Δ	Lethal

Decoding site
Conserved nucleotides

E. coli *H. sapiens/*yeast

A^{1493} -------- A^{1756}

A^{1492} -------- A^{1755}

G^{530} -------- G^{577}

Mutagenesis of the yeast decoding site residues led to **lethal** phonotypes

Result:

G577, A1755 and A1756 are **essential** for cell viability

(Fan-Minogue and Bedwell, RNA (2008)

Mutagenesis or deletion of non-conserved nucleotides

E. coli residue	*S. cerevisiae* mutation	Viability[a]
WT	WT	Viable (++)
A1408	G1645A	Viable (++)
	G1645C	Viable (++)
	G1645U	Lethal
	G1645Δ	Lethal
G1491	A1754G	Viable (++)
A1408/G1491	G1645A/A1754G	Viable (++)

Decoding site
Non-conserved nucleotides

E. coli *Uomo/lievito*

G^{1491} ----//-- A^{1754}

A^{1408} ----//-- G^{1645}

(Fan-Minogue and Bedwell, RNA (2008)

Mutagenesis or deletion of non-conserved nucleotides

E. coli residue	*S. cerevisiae* mutation	Viability[a]
WT	WT	Viable (++)
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	G1645C	Viable (++)
	G1645U	Lethal
	G1645Δ	Lethal
G1491 →	A1754G	Viable (++)
A1408/G1491	G1645A/A1754G	Viable (++)

Decoding site
Non-conserved nucleotides

E. coli *Uomo/lievito*

G^{1491} ----//-- A^{1754}

A^{1408} ----//-- G^{1645}

Eukaryotic → Prokaryotic

conversion of yeast decoding site

(Fan-Minogue and Bedwell, RNA (2008)

Mutagenesis or deletion of non-conserved nucleotides

E. coli residue	*S. cerevisiae* mutation	Viability[a]
WT	WT	Viable (++)
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	G1645U	Lethal
	G1645Δ	Lethal
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A1408/G1491	G1645A/A1754G	Viable (++)

Decoding site
Non-conserved nucleotides

E. coli *Uomo/lievito*

G^{1491} ---//--- A^{1754}

A^{1408} ---//--- G^{1645}

Eukaryotic → Prokaryotic
conversion of yeast decoding site

Single G→A (G1645A) and A→G (A1754G) or double (G1645A/A1754G) substitutions of non-conserved A/G nucleotides led to viable phenotypes

Result:

The **non-conserved nucleotides**, involved in aminoglycoside binding, are **not essential for cell viability** and **ribosome activity**

(Fan-Minogue and Bedwell, RNA (2008)

Decoding site mutations at non-conserved nucleotides alter aminoglycoside resistance in yeast

TABLE 2. Aminoglycoside sensitivity of *S. cerevisiae* decoding-site mutants

Aminoglycoside	*S. cerevisiae* MIC[a] in strains with indicated 18S rRNA mutation (μg/mL)					*E. coli* MIC (μg/mL)[b] WT
	WT	G1645A	G1645C	A1754G	G1645A/A1754G	
Kanamycin A	> 5000	25	1000	5000	3	2.5
Neomycin	> 5000	25	1000	5000	3	5
Paromomycin	> 1500	200	> 1500	25	3	5
G418	50	15	> 50	5	3	2.5

[a]MIC, minimal inhibitory concentration.

(Fan-Minogue and Bedwell, RNA (2008)

Decoding site mutations at non-conserved nucleotides alter aminoglycoside resistance in yeast

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Paromomycin	> 1500	200	> 1500	25	3	5
G418	50	15	> 50	5	3	2.5

[a]MIC, minimal inhibitory concentration.

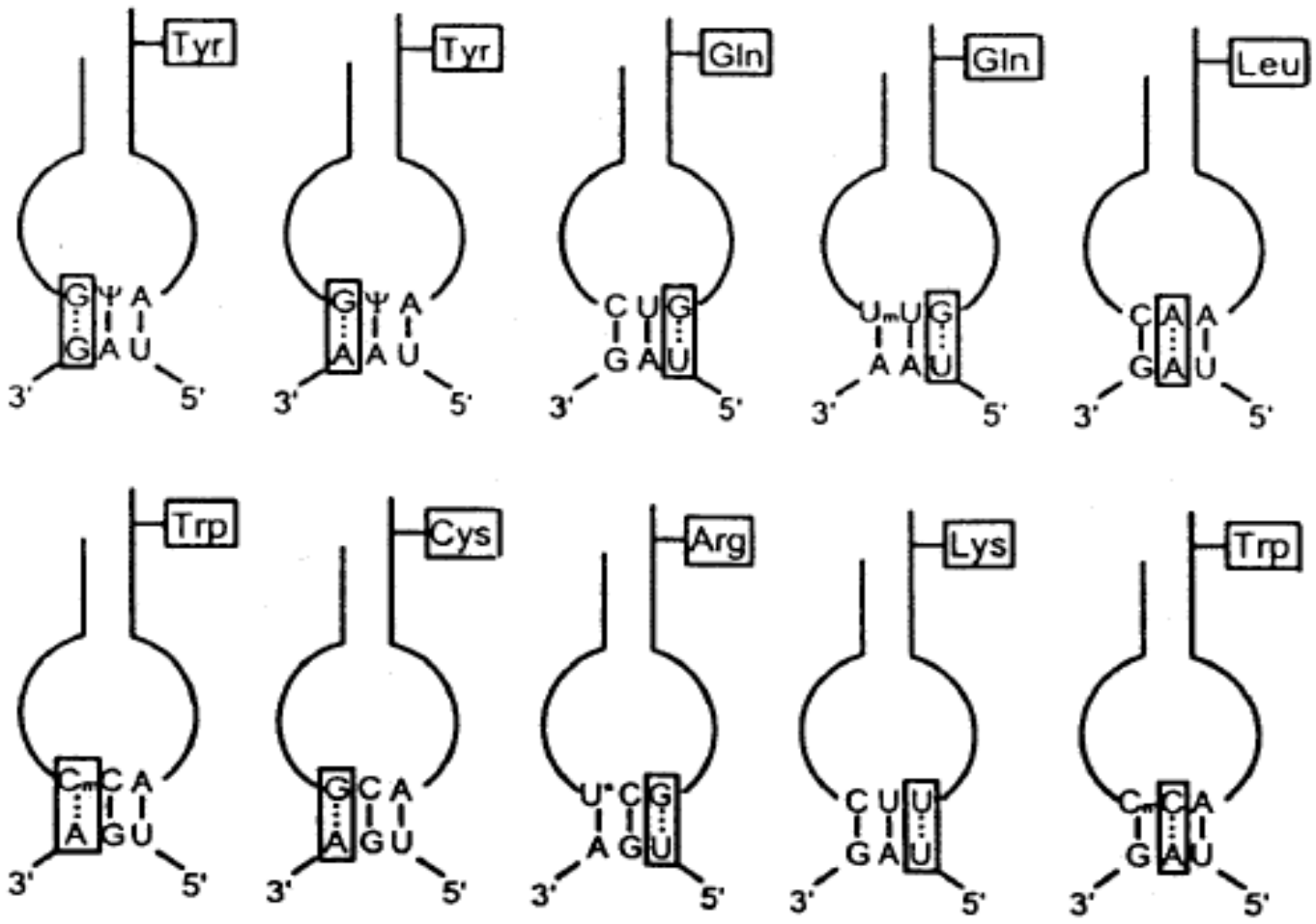
The double mutant G1645A/A1754G showed a sensivity to aminoglycosides similar to that of prokaryotic (*E. coli*) decoding site

Results indicate that nucleotide divergence at both G1645 and A1754 is responsible for the **differential sensitivity to aminoglycosides** observed **between prokaryotes and eukaryotes.**

(Fan-Minogue and Bedwell, RNA (2008)

tRNA soppressori (suppressor tRNAs)

Presenti nelle cellule, sfruttano gli appaiamenti non standard tra codone e anticodone



UAG/UAA

tRNA^{Tyr}

tRNA^{Gln}

tRNA^{Leu}

tRNA^{Lys}

UGA

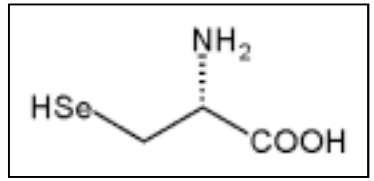
tRNA^{Trp}

tRNA^{Cys}

tRNA^{Arg}

Incorporation of selenocysteine, the 21st amino acid, occurs at in-frame UGA codons

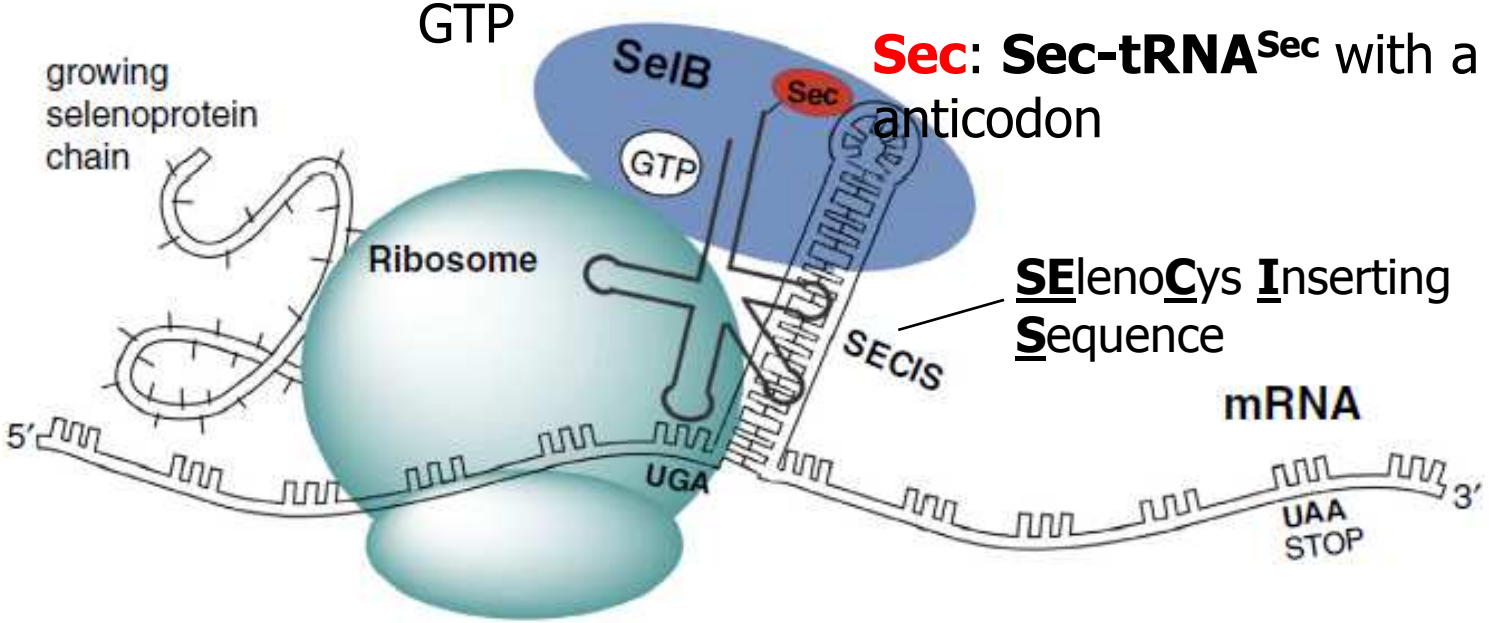
- Whenever a stop codon enters the ribosomal A site, a competition occurs between the class I release factor(s) and near-cognate tRNAs (that can base pair at 2 of the 3 nucleotides of the stop codon).
- The release factor normally wins this competition >99% of the time, but this efficiency can be reduced by the sequence context around the stop codon, the relative level of the release factor, and the presence of downstream elements that can stimulate suppression.
- Selenocysteine incorporation requires a selenocysteine insertion element (SECIS).
- In eubacteria, the specialized translation elongation factor SelB binds both the SECIS just downstream of the SECIS and $tRNA^{(ser)sec}$.
- In eukaryotes, the SECIS is located in the 3´-UTR of the mRNA. Association of mSelB (also known as eEFsec) to the SECIS element requires the adaptor protein SBP2.



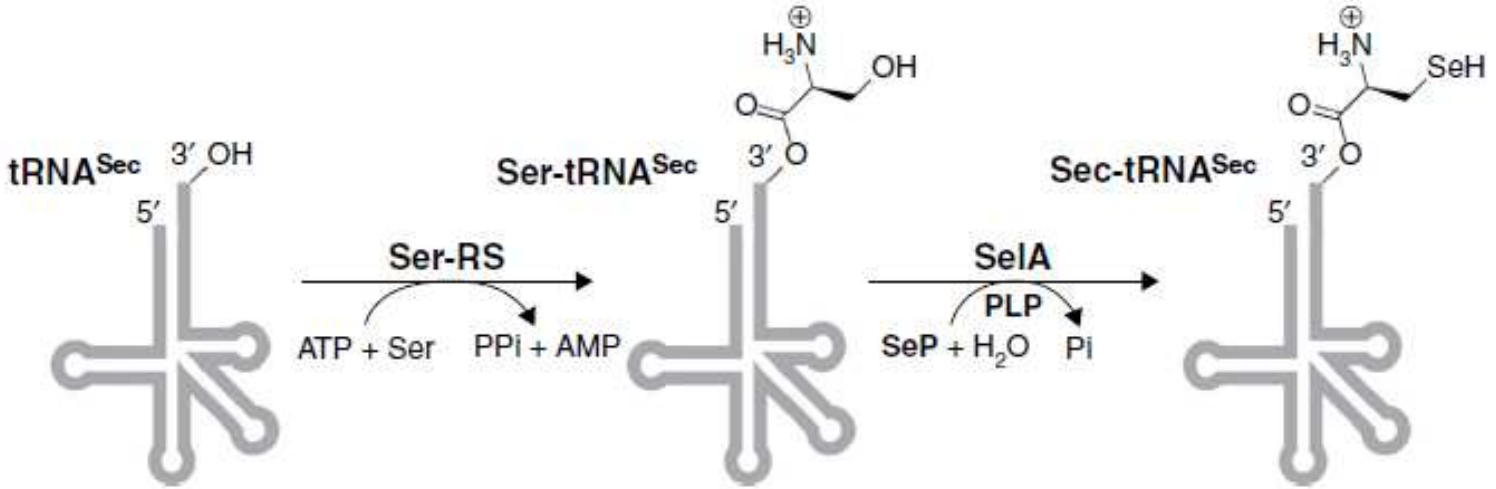
Bacterial selenoprotein biosynthesis

SelB: elongation factor carrying Sec and GTP

Sec: **Sec-tRNA^{Sec}** with a UCA anticodon



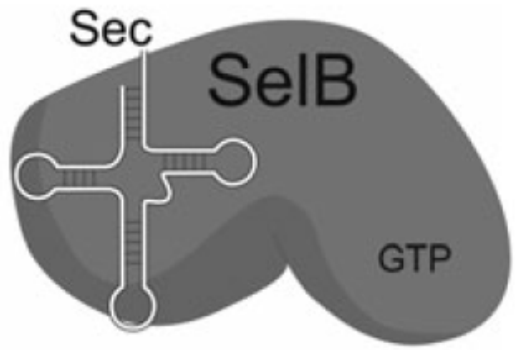
SEleno**C**ys **I**nserting **S**equence



Metanis *et al.*, Current Opinion in Chemical Biology, 2014

Selenocysteine is the 21st aminoacid in the genetic code

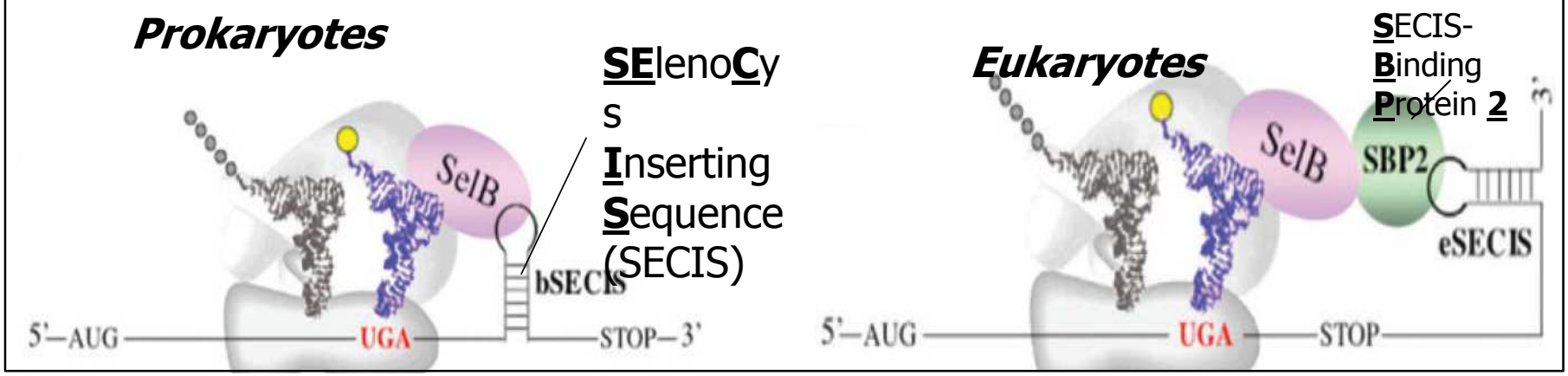
- Structurally identical to Cys, but with the thiol group replaced by a selenol group.
- Discovered as a unique amino acid in 1976
- Found to be co-translationally inserted into growing peptides in 1986
- Incorporated into proteins by translational redefinition of **UGA codons**.



Sec: **Sec-tRNA^{Sec}** with a UCA anticodon complementary to UGA stop codon

SelB: specialized translation elongation factor carrying Sec and GTP

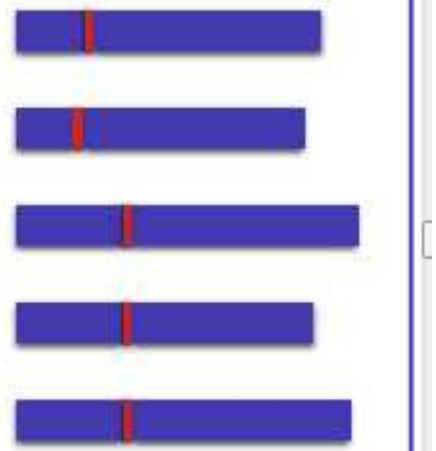
Selenocysteine decoding and insertion during translation



Cobucci-Ponzano *et al.*, Molecular Microbiology, 2005

The human selenoproteome

Selenoprotein	Function	Protein size
Glutathione peroxidase 1	Cytosolic glutathione peroxidase	201
Glutathione peroxidase 2	Gastrointestinal glutathione peroxidase	190
Glutathione peroxidase 3	Plasma glutathione peroxidase	226
Glutathione peroxidase 4	Phospholipid hydroperoxide glutathione peroxidase	197
Glutathione peroxidase 6	Olfactory glutathione peroxidase	221
Selenoprotein W	Unknown	87
Selenoprotein T	Unknown	182
Selenoprotein H	Unknown	116
Selenoprotein V	Unknown. Testis-specific expression	346
Selenoprotein I	Unknown	397



Hatfield *et al.*, Trends in Biochemical Sciences, 2014